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Attorney Docket No. PPL-1 - Reissue

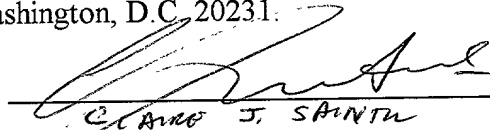
Applicants : Ian Garner et al.
For : PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS

EXPRESS MAIL CERTIFICATION

"Express Mail" mailing label number EI187448225US

Date of Deposit January 15, 1999

I hereby certify that this transmittal letter and the other papers and fees identified in this transmittal letter as being transmitted herewith are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and are addressed to the Hon. Assistant Commissioner for Patents, Washington, D.C. 20231.


C. J. SMITH

Hon. Assistant Commissioner for Patents
Washington, D.C. 20231

TRANSMITTAL LETTER FOR
REISSUE PATENT APPLICATION

Sir:

This is a request for filing a reissue application of United States Patent No. 5,639,940, issued June 17, 1997 entitled, PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS.

Transmitted herewith for filing are the ☒ specification (18 pages); ☒ claims (34); ☒ abstract; ☒ sequence listings (27); ☒ executed Reissue Declaration and Power of Attorney; ☒ executed Parker Declaration in Support of Reissue; ☒ Order for Title Report; ☒ Offer to Surrender Original Letters Patent; and ☒ Consent of Assignees (2) for the above-identified Patent application.

Also transmitted herewith are:

☒ 5 sheets of:

01/15/99
jc526 U.S. PTO

jc542 U.S. PTO
09/232488
01/15/99

09232488 041599

☒ Formal drawings.

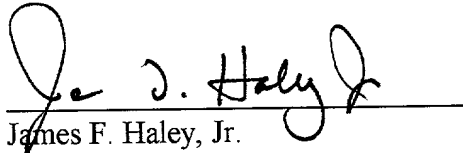
The filing fee has been calculated as shown below on the basis of the reissue claims filed herewith:

FOR	NUMBER EXTRA		RATE		FEE
BASIC FEE					\$760.00
TOTAL INDEPENDENT CLAIMS IN EXCESS OF THE NUMBER OF INDEPENDENT CLAIMS IN ORIGINAL PATENT	1	x	\$ 78	=	\$78.00
REISSUE CLAIMS IN EXCESS OF 20 AND IN EXCESS OF ORIGINAL CLAIMS	1	x	\$18	=	\$ 18.00
TOTAL					<u>\$ 856.00</u>

☒ A check in the amount of \$ 856.00 in payment of the filing fee is transmitted herewith.



The Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 C.F.R. § 1.16 in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.



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66940 884666

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reissue Application : Not yet assigned
 Serial No. : Not yet assigned
 Filed : January 15, 1999
 Patent : 5,639,940
 Patentee : Ian Garner, Michael A. Dalrymple, * Donna E. Prunkard
 and Donald C. Foster
 Assignee : Pharmaceutical Proteins, Ltd., and ZymoGenetics, Inc.
 Issued : June 17, 1997
 Application : 08/206,176
 Filed : March 3, 1994
 For : PRODUCTION OF FIBRINOGEN IN TRANSGENIC
 ANIMALS

Hon. Assistant Commissioner for Patents
 Washington, D.C. 20231

CONSENT OF ASSIGNEE TO REISSUE APPLICATION

Sir:

ZymoGenetics, Inc., a co-assignee of the above-identified '940 patent, by virtue of an August 19, 1994 assignment of United States patent application 08/206,176, filed March 3, 1994, from Donna E. Prunkard and Donald C. Foster, recorded at Reel 7166, Frame 0921, hereby consents to the above-identified reissue of the '940 Patent.

* The '940 patent incorrectly printed the middle initial of Dr. Dalrymple as "L."

Pursuant to 37 C.F.R. §§ 1.172 and 3.73(b), the undersigned hereby states and certifies that:

1. I am an officer of assignee corporation and am authorized to act on behalf of assignee corporation with respect to the above identified '940 Patent and this reissue thereof, and

2. The relevant evidentiary documents have been reviewed and, to the best of my knowledge and belief, an undivided share of the title to the '940 Patent is in the assignee.

ZYMOGENETICS, INC.,

By: Shirley Campos

Name: SHIRLEY Campos

Title: Sr. V.P. Finance + Admin.

Date

Reissue Application	:	Not yet assigned
Serial No.	:	Not yet assigned
Filed	:	January 15, 1999
Patent	:	5,639,940
Patentee	:	Ian Garner, Michael A. Dalrymple,* Donna E. Prunkard and Donald C. Foster
Assignee	:	Pharmaceutical Proteins, Ltd., and ZymoGenetics, Inc
Issued	:	June 17, 1997
Application	:	08/206,176
Filed	:	March 3, 1994
For	:	PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS

CONSENT OF ASSIGNEE TO REISSUE APPLICATION

Pharmaceutical Proteins, Ltd., a co-assignee of the above-identified '940 Patent, by virtue of an August 12, 1994 assignment of United States patent application 08/206,176, filed March 3, 1994, from Ian Garner and Michael A. Dalrymple, recorded at Reel 7166. Frame 0931, hereby consents to the above-identified reissue of the '940 Patent.

* The '940 patent incorrectly printed the middle initial of Dr. Dalrymple as "L".

Pursuant to 37 C.F.R. §§ 1.172 and 3.73(b), the undersigned hereby states and certifies that:

1. I am an officer of assignee corporation and am authorized to act on behalf of assignee corporation with respect to the above identified '940 Patent and this reissue thereof, and

2. The relevant evidentiary documents have been reviewed and, to the best of my knowledge and belief, an undivided share of the title to the '940 Patent is in the assignee.

PHARMACEUTICAL PROTEINS, LTD.

15/1/99
Date

By: Alan Coleman

Name: ALAN COLMAN

Title: RESEARCH DIRECTOR

PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS

BACKGROUND OF THE INVENTION

The final step in the blood coagulation cascade is the thrombin-catalyzed conversion of the soluble plasma protein fibrinogen to insoluble fibrin. Thrombin cleaves a small peptide (fibrinopeptide A) from one of the three component chains (the A α -chain) of fibrinogen. Fibrin monomers subsequently polymerize and are cross-linked by activated factor XIII to form a stable clot.

Fibrinogen is a key component of biological tissue glues (see, e.g., U.S. Pat. Nos. 4,377,572 and 4,442,655), which mimic the formation of natural blood clots to promote hemostasis and repair damaged tissue. Tissue glues provide an adjunct or alternative to sutures, staples and other mechanical means for wound closure. However, the principal ingredients of these products (fibrinogen, factor XIII and thrombin) are prepared from pooled human plasma by cryoprecipitation (e.g. U.S. Pat. Nos. 4,377,572; 4,362,567; 4,909,251) or ethanol precipitation (e.g. U.S. Pat. No. 4,442,655) or from single donor plasma (e.g. U.S. Pat. No. 4,627,879; Spotnitz et al., *Am. Surg.* 55: 166-168, 1989). The resultant fibrinogen/factor XIII preparation is mixed with bovine thrombin immediately before use to convert the fibrinogen to fibrin and activate the factor XIII, thus initiating coagulation of the adhesive.

Commercially available adhesives are of pooled plasma origin. Because blood-derived products have been associated with the transmission of human immunodeficiency virus (HIV), hepatitis virus and other etiologic agents, the acceptance and availability of such adhesives is limited. At present they are not approved for use in the United States.

While the use of autologous plasma reduces the risk of disease transmission, autologous adhesives can only be used in elective surgery when the patient is able to donate the necessary blood in advance.

As noted above, fibrinogen consists of three polypeptide chains, each of which is present in two copies in the assembled molecule. These chains, designated the A α , B β and γ -chains, are coordinately expressed, assembled and secreted by the liver. While it might be expected that recombinant DNA technology could provide an alternative to the isolation of fibrinogen from plasma, this goal has proven to be elusive. The three fibrinogen chains have been individually expressed in *E. coli* (Lord, *DNA* 4: 33-38, 1985; Bolyard and Lord, *Gene* 66: 183-192, 1988; Bolyard and Lord, *Blood* 73: 1202-1206), but functional fibrinogen has not been produced in a prokaryotic system. Expression of biologically competent fibrinogen in yeast has not been reported. Cultured transfected mammalian cells have been used to express biologically active fibrinogen (Farrell et al., *Blood* 74: 55a, 1989; Hartwig and Danishefsky, *J. Biol. Chem.* 266: 6578-6585, 1991; Farrell et al., *Biochemistry* 30: 9414-9420, 1991), but expression levels have been so low that production of recombinant fibrinogen in commercial quantities is not feasible. Experimental evidence suggests that lower transcription rates in cultured cells as compared to liver may be a factor in the low expression rates achieved to date, but increasing the amount of fibrinogen chain mRNA in transfected BHK cells did not produce corresponding increases in fibrinogen protein secretion (Prunkard and Foster, XIV Congress of the International Society on Thrombosis and Haemostasis, 1993). These latter results suggest that proper assembly and processing of fibrinogen involves tissue-specific mechanisms not present in common laboratory cell lines.

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SUMMARY OF THE INVENTION

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ologous DNA segments encoding $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen. Within a related aspect, the invention provides a transgenic non-human female mammal that produces recoverable amounts of human fibrinogen in its milk.

Within another aspect, the invention provides a method for producing a transgenic offspring of a mammal comprising the steps of (a) providing a first DNA segment encoding a fibrinogen $\text{A}\alpha$ chain, a second DNA segment encoding a fibrinogen $\text{B}\beta$ chain, and a third DNA segment encoding a fibrinogen γ chain, wherein each of said first, second and third segments is operably linked to additional DNA segments required for its expression in a mammary gland of a host female mammal and secretion into milk of the host female mammal; (b) introducing the DNA segments into a fertilized egg of a mammal of a non-human species; (c) inserting the egg into an oviduct or uterus of a female of the non-human species to obtain an offspring carrying the first, second and third DNA segments. In a related aspect, the invention provides non-human mammals produced according to this process.

Within an additional aspect, the invention provides a non-human mammal carrying its germline DNA segments encoding heterologous $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen, wherein female progeny of the mammal express the DNA segments in a mammary gland to produce biocompetent fibrinogen.

These and other aspects of the invention will become evident to the skilled practitioner upon reference to the following detailed description and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the subcloning of a human fibrinogen $\text{A}\alpha$ chain DNA sequence.

FIG. 2 is a partial restriction map of the vector Zem228. Symbols used are MT-1p, mouse metallothionein promoter; SV40t, SV40 terminator; and SV40p, SV40 promoter.

FIG. 3 illustrates the subcloning of a human fibrinogen $\text{B}\beta$ chain DNA sequence.

FIG. 4 illustrates the subcloning of a human fibrinogen γ chain DNA sequence.

FIG. 5 is a partial restriction map of the vector Zem219b. Symbols used are MT-1p, mouse metallothionein promoter; hGHt, human growth hormone terminator; SV40p, SV40 promoter; DHFR, dihydrofolate reductase gene; and SV40t, SV40 terminator.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention in detail, it will be helpful to define certain terms used herein:

As used herein, the term "biocompetent fibrinogen" is used to denote fibrinogen that polymerizes when treated with thrombin to form insoluble fibrin.

The term "egg" is used to denote an unfertilized ovum, a fertilized ovum prior to fusion of the pronuclei or an early stage embryo (fertilized ovum with fused pronuclei).

A "female mammal that produces milk containing biocompetent fibrinogen" is one that, following pregnancy and delivery, produces, during the lactation period, milk containing recoverable amounts of biocompetent fibrinogen. Those skilled in the art will recognize that such animals will produce milk, and therefore the fibrinogen, discontinuously.

The term "progeny" is used in its usual sense to include children and descendants.

The term "heterologous" is used to denote genetic material originating from a different species than that into which it has been introduced, or a protein produced from such genetic material.

- 5 Within the present invention, transgenic animal technology is employed to produce fibrinogen within the mammary glands of a host female mammal. Expression in the mammary gland and subsequent secretion of the protein of interest into the milk overcomes many difficulties encountered in isolating proteins from other sources. Milk is readily
10 collected, available in large quantities, and well characterized biochemically. Furthermore, the major milk proteins are present in milk at high concentrations (from about 1 to 15 g/l).
- 15 From a commercial point of view, it is clearly preferable to use as the host a species that has a large milk yield. While smaller animals such as mice and rats can be used (and are preferred at the proof-of-concept stage), within the present invention it is preferred to use livestock mammals including,
20 but not limited to, pigs, goats, sheep and cattle. Sheep are particularly preferred due to such factors as the previous history of transgenesis in this species, milk yield, cost and the ready availability of equipment for collecting sheep milk. See WO 88/00239 for a comparison of factors influencing the choice of host species. It is generally desirable to
25 select a breed of host animal that has been bred for dairy use, such as East Friesland sheep, or to introduce dairy stock by breeding of the transgenic line at a later date. In any event, animals of known, good health status should be used.
- 30 Fibrinogen produced according to the present invention may be human fibrinogen or fibrinogen of a non-human animal. For medical uses, it is preferred to employ proteins native to the patient. The present invention thus provides fibrinogen for use in both human and veterinary medicine.
- 35 Cloned DNA molecules encoding the component chains of human fibrinogen are disclosed by Rixon et al. (*Biochem.* 22: 3237, 1983), Chung et al. (*Biochem.* 22: 3244, 1983), Chung et al. (*Biochem.* 22: 3250, 1983), Chung et al. (*Adv. Exp. Med. Biol.* 281: 39-48, 1990) and Chung et al. (*Ann.*
40 *NY Acad. Sci.* 408: 449-456, 1983). Bovine fibrinogen clones are disclosed by Brown et al. (*Nuc. Acids Res.* 17: 6397, 1989) and Chung et al. (*Proc. Natl. Acad. Sci. USA* 78: 1466-1470, 1981). Other mammalian fibrinogen clones are disclosed by Murakawa et al. (*Thromb. Haemost.* 69:
45 351-360, 1993). Representative sequences of human α , β and γ chain genes are shown in SEQ ID NOS: 1, 3 and 5, respectively. Those skilled in the art will recognize that allelic variants of these sequences will exist; that additional variants can be generated by amino acid substitution,
50 deletion, or insertion; and that such variants are useful within the present invention. In general, it is preferred that any engineered variants comprise only a limited number of amino acid substitutions, deletions, or insertions, and that any substitutions are conservative. Thus, it is preferred to
55 produce fibrinogen chain polypeptides that are at least 90%, preferably at least 95, and more preferably 99% or more identical in sequence to the corresponding native chains. The term " γ chain" is meant to include the alternatively spliced γ chain of fibrinogen (Chung et al., *Biochem.* 23:
60 4232-4236, 1984). A human γ chain amino acid sequence is shown in SEQ ID NO: 6. The shorter γ chain is produced by alternative splicing at nucleotides 9511 and 10054 of SEQ ID NO: 5, resulting in translation terminating after nucleotide 10065 of SEQ ID NO: 5.
- 65 To obtain expression in the mammary gland, a transcription promoter from a milk protein gene is used. Milk protein genes include those genes encoding caseins, beta-

lactoglobulin (BLG), α -lactalbumin, and whey acidic protein. The beta-lactoglobulin promoter is preferred. In the case of the ovine beta-lactoglobulin gene, a region of at least the proximal 406 bp of 5' flanking sequence of the ovine BLG gene (contained within nucleotides 3844 to 4257 of SEQ ID NO:7) will generally be used. Larger portions of the 5' flanking sequence, up to about 5 kbp, are preferred. A larger DNA segment encompassing the 5' flanking promoter region and the region encoding the 5' non-coding portion of the beta-lactoglobulin gene (contained within nucleotides 1 to 4257 of SEQ ID NO:7) is particularly preferred. See Whitelaw et al., *Biochem J.* 28: 31-39, 1992. Similar fragments of promoter DNA from other species are also suitable.

Other regions of the beta-lactoglobulin gene may also be incorporated in constructs, as may genomic regions of the gene to be expressed. It is generally accepted in the art that constructs lacking introns, for example, express poorly in comparison with those that contain such DNA sequences (see Brinster et al., *Proc. Natl. Acad. Sci. USA* 85: 836-840, 1988; Palmiter et al., *Proc. Natl. Acad. Sci. USA* 88: 478-482, 1991; Whitelaw et al., *Transgenic Res.* 1: 3-13, 1991; WO 89/01343; WO 91/02318). In this regard, it is generally preferred, where possible, to use genomic sequences containing all or some of the native introns of a gene encoding the protein or polypeptide of interest. Within certain embodiments of the invention, the further inclusion of at least some introns from the beta-lactoglobulin gene is preferred. One such region is a DNA segment which provides for intron splicing and RNA polyadenylation from the 3' non-coding region of the ovine beta-lactoglobulin gene. When substituted for the natural 3' non-coding sequences of a gene, this ovine beta-lactoglobulin segment can both enhance and stabilize expression levels of the protein or polypeptide of interest. Within other embodiments, the region surrounding the initiation ATG of one or more of the fibrinogen sequences is replaced with corresponding sequences from a milk specific protein gene. Such replacement provides a putative tissue-specific initiation environment to enhance expression. It is convenient to replace the entire fibrinogen chain pre-pro and 5' non-coding sequences with those of, for example, the BLG gene, although smaller regions may be replaced.

For expression of fibrinogen, DNA segments encoding each of the three component polypeptide chains of fibrinogen are operably linked to additional DNA segments required for their expression to produce expression units. Such additional segments include the above-mentioned milk protein gene promoter, as well as sequences which provide for termination of transcription and polyadenylation of mRNA. The expression units will further include a DNA segment encoding a secretion signal operably linked to the segment encoding the fibrinogen polypeptide chain. The secretion signal may be a native fibrinogen secretion signal or may be that of another protein, such as a milk protein. The term "secretion signal" is used herein to denote that portion of a protein that directs it through the secretory pathway of a cell to the outside. Secretion signals are most commonly found at the amino-termini of proteins. See, for example, von Heinje, *Nuc. Acids Res.* 14: 4683-4690, 1986; and Meade et al., U.S. Pat. No. 4,873,316, which are incorporated herein by reference.

Construction of expression units is conveniently carried out by inserting a fibrinogen chain sequence into a plasmid or phage vector containing the additional DNA segments, although the expression unit may be constructed by essentially any sequence of ligations. It is particularly convenient

to provide a vector containing a DNA segment encoding a milk protein and to replace the coding sequence for the milk protein with that of a fibrinogen chain (including a secretion signal), thereby creating a gene fusion that includes the expression control sequences of the milk protein gene. In any event, cloning of the expression units in plasmids or other vectors facilitates the amplification of the fibrinogen sequences. Amplification is conveniently carried out in bacterial (e.g. *E. coli*) host cells, thus the vectors will typically include an origin of replication and a selectable marker functional in bacterial host cells.

In view of the size of the fibrinogen chain genes it is most practical to prepare three separate expression units, mix them, and introduce the mixture into the host. However, those skilled in the art will recognize that other protocols may be followed. For example, expression units for the three chains can be introduced individually into different embryos to be combined later by breeding. In a third approach, the three expression units can be linked in a single suitable vector, such as a yeast artificial chromosome or phage P1 clone. Coding sequences for two or three chains can be combined in polycistronic expression units (see, e.g., Levinson et al., U.S. Pat. No. 4,713,339).

The expression unit(s) is(are) then introduced into fertilized eggs (including early-stage embryos) of the chosen host species. Introduction of heterologous DNA can be accomplished by one of several routes, including microinjection (e.g. U.S. Pat. No. 4,873,191), retroviral infection (Jaenisch, *Science* 240: 1468-1474, 1988) or site-directed integration using embryonic stem (ES) cells (reviewed by Bradley et al., *Bio/Technology* 10: 534-539, 1992). The eggs are then implanted into the oviducts or uteri of pseudopregnant females and allowed to develop to term. Offspring carrying the introduced DNA in their germ line can pass the DNA on to their progeny in the normal, Mendelian fashion, allowing the development of transgenic herds. General procedures for producing transgenic animals are known in the art. See, for example, Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1986; Simons et al., *Bio/Technology* 6: 179-183, 1988; Wall et al., *Biol. Reprod.* 32: 645-651, 1985; Buhler et al., *Bio/Technology* : 140-143, 1990; Ebert et al., *Bio/Technology* : 835-838, 1991; Krimpenfort et al., *Bio/Technology* 9: 844-847, 1991; Wall et al., *J. Cell. Biochem.* 49: 113-120, 1992; and WIPO publications WO 88/00239, WO 90/05188, WO 92/11757; and GB 87/00458, which are incorporated herein by reference. Techniques for introducing foreign DNA sequences into mammals and their germ cells were originally developed in the mouse. See, e.g., Gordon et al., *Proc. Natl. Acad. Sci. USA* 77: 7380-7384, 1980; Gordon and Ruddle, *Science* 214: 1244-1246, 1981; Palmiter and Brinster, *Cell* 41: 343-345, 1985; Brinster et al., *Proc. Natl. Acad. Sci. USA* 82: 4438-4442, 1985; and Hogan et al. (ibid.). These techniques were subsequently adapted for use with larger animals, including livestock species (see e.g., WIPO publications WO 88/00239, WO 90/05188, and WO 92/11757; and Simons et al., *Bio/Technology* 6: 179-183, 1988). To summarize, in the most efficient route used to date in the generation of transgenic mice or livestock, several hundred linear molecules of the DNA of interest are injected into one of the pro-nuclei of a fertilized egg. Injection of DNA into the cytoplasm of a zygote can also be employed.

It is preferred to obtain a balanced expression of each fibrinogen chain to allow for efficient formation of the mature protein. Ideally, the three expression units should be on the same DNA molecule for introduction into eggs. This approach, however, may generate technical problems at, for

example, the injection and manipulation stages. For example, the size of fibrinogen expression units may necessitate the use of yeast artificial chromosomes (YACs) or phage P1 to amplify and manipulate the DNA prior to injection. If this approach is followed, segments of DNA to be injected, containing all three expression units, would be very large, thus requiring modification of the injection procedure using, for example, larger bore needles. In a more simple approach, a mixture of each individual expression unit is used. It is preferred to combine equimolar amounts of the three expression units, although those skilled in the art will recognize that this ratio may be varied to compensate for the characteristics of a given expression unit. Some expression, generally a reduced level, will be obtained when lesser molar amounts of one or two chains are used, and expression efficiencies can generally be expected to decline in approximate proportion to the divergence from the preferred equimolar ratio. In any event, it is preferred to use a mixture having a ratio of $\alpha\alpha:\beta\beta:\gamma$ expression units in the range of 0.5-1:0.5-1:0.5-1. When the ratio is varied from equimolar, it is preferred to employ relatively more of the $\beta\beta$ expression unit. Alternatively, one or a mixture of two of the expression units is introduced into individual eggs. However, animals derived by this approach will express only one or two fibrinogen chains. To generate an intact fibrinogen molecule by this approach requires a subsequent breeding program designed to combine all three expression units in individuals of a group of animals.

In general, female animals are superovulated by treatment with follicle stimulating hormone, then mated. Fertilized eggs are collected, and the heterologous DNA is injected into the eggs using known methods. See, for example, U.S. Pat. No. 4,873,191; Gordon et al., *Proc. Natl. Acad. Sci. USA* 77: 7380-7384, 1980; Gordon and Ruddle, *Science* 214: 1244-1246, 1981; Palmiter and Brinster, *Cell* 41: 343-345, 1985; Brinster et al., *Proc. Natl. Acad. Sci. USA* 82: 4438-4442, 1985; Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1986; Simons et al. *Bio/Technology* 6: 179-183, 1988; Wall et al., *Biol. Reprod.* 32: 645-651, 1985; Buhler et al., *Bio/Technology* 8: 140-143, 1990; Ebert et al., *Bio/Technology* 9: 835-838, 1991; Krimpenfort et al., *Bio/Technology* 9: 844-847, 1991; Wall et al., *J. Cell. Biochem.* 49: 113-120, 1992; WIPO publications WO 88/00239, WO 90/05118, and WO 92/11757; and GB 87/00458, which are incorporated herein by reference.

For injection into fertilized eggs, the expression units are removed from their respective vectors by digestion with appropriate restriction enzymes. For convenience, it is preferred to design the vectors so that the expression units are removed by cleavage with enzymes that do not cut either within the expression units or elsewhere in the vectors. The expression units are recovered by conventional methods, such as electro-elution followed by phenol extraction and ethanol precipitation, sucrose density gradient centrifugation, or combinations of these approaches.

DNA is injected into eggs essentially as described in Hogan et al., *ibid.* In a typical injection, eggs in a dish of an embryo culture medium are located using a stereo zoom microscope ($\times 50$ or $\times 63$ magnification preferred). Suitable media include Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid) or bicarbonate buffered media such as M2 or M16 (available from Sigma Chemical Co., St. Louis, USA) or synthetic oviduct medium (disclosed below). The eggs are secured and transferred to the center of a glass slide on an injection rig using, for example, a drummond pipette complete with capillary tube. Viewing at lower (e.g. $\times 4$)

magnification is used at this stage. Using the holding pipette of the injection rig, the eggs are positioned centrally on the slide. Individual eggs are sequentially secured to the holding pipette for injection. For each injection process, the holding pipette/egg is positioned in the center of the viewing field. The injection needle is then positioned directly below the egg. Preferably using $\times 40$ Nomarski objectives, both manipulator heights are adjusted to focus both the egg and the needle. The pronuclei are located by rotating the egg and adjusting the holding pipette assembly as necessary. Once the pronucleus has been located, the height of the manipulator is altered to focus the pronuclear membrane. The injection needle is positioned below the egg such that the needle tip is in a position below the center of the pronucleus. The position of the needle is then altered using the injection manipulator assembly to bring the needle and the pronucleus into the same focal plane. The needle is moved, via the joy stick on the injection manipulator assembly, to a position to the right of the egg. With a short, continuous jabbing movement, the pronuclear membrane is pierced to leave the needle tip inside the pronucleus. Pressure is applied to the injection needle via the glass syringe until the pronucleus swells to approximately twice its volume. At this point, the needle is slowly removed. Reverting to lower (e.g. $\times 4$) magnification, the injected egg is moved to a different area of the slide, and the process is repeated with another egg.

After the DNA is injected, the eggs may be cultured to allow the pronuclei to fuse, producing one-cell or later stage embryos. In general, the eggs are cultured at approximately the body temperature of the species used in a buffered medium containing balanced salts and serum. Surviving embryos are then transferred to pseudopregnant recipient females, typically by inserting them into the oviduct or uterus, and allowed to develop to term. During embryogenesis, the injected DNA integrates in a random fashion in the genomes of a small number of the developing embryos.

Potential transgenic offspring are screened via blood samples and/or tissue biopsies. DNA is prepared from these samples and examined for the presence of the injected construct by techniques such as polymerase chain reaction (PCR; see Mullis, U.S. Pat. No. 4,683,202) and Southern blotting (Southern, *J. Mol. Biol.* 98:503, 1975; Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1982). Founder transgenic animals, or G0s, may be wholly transgenic, having transgenes in all of their cells, or mosaic, having transgenes in only a subset of cells (see, for example, Wilkie et al., *Develop. Biol.* 118: 9-18, 1986). In the latter case, groups of germ cells may be wholly or partially transgenic. In the latter case, the number of transgenic progeny from a founder animal will be less than the expected 50% predicted from Mendelian principles. Founder G0 animals are grown to sexual maturity and mated to obtain offspring, or G1s. The G1s are also examined for the presence of the transgene to demonstrate transmission from founder G0 animals. In the case of male G0s, these may be mated with several non-transgenic females to generate many offspring. This increases the chances of observing transgene transmission. Female G0 founders may be mated naturally, artificially inseminated or superovulated to obtain many eggs which are transferred to surrogate mothers. The latter course gives the best chance of observing transmission in animals having a limited number of young. The above-described breeding procedures are used to obtain animals that can pass the DNA on to subsequent generations of offspring in the normal, Mendelian fashion, allowing the development of, for example, colonies (mice), flocks (sheep), or herds (pigs, goats and cattle) of transgenic animals.

The milk from lactating G0 and G1 females is examined for the expression of the heterologous protein using immunological techniques such as ELISA (see Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and Western blotting (Towbin et al., *Proc. Natl. Acad. Sci. USA* 76: 4350-4354, 1979). For a variety of reasons known in the art, expression levels of the heterologous protein will be expected to differ between individuals.

A satisfactory family of animals should satisfy three criteria: they should be derived from the same founder G0 animal; they should exhibit stable transmission of the transgene; and they should exhibit stable expression levels from generation to generation and from lactation to lactation of individual animals. These principles have been demonstrated and discussed (Carver et al., *Bio/Technology* 11: 1263-1270, 1993). Animals from such a suitable family are referred to as a "line." Initially, male animals, G0 or G1, are used to derive a flock or herd of producer animals by natural or artificial insemination. In this way, many female animals containing the same transgene integration event can be quickly generated from which a supply of milk can be obtained.

The fibrinogen is recovered from milk using standard practices such as skimming, precipitation, filtration and protein chromatography techniques.

Fibrinogen produced according to the present invention is useful within human and veterinary medicine, such as in the formulation of surgical adhesives. Adhesives of this type are known in the art. See, for example, U.S. Pat. Nos. 4,377,572; 4,442,655; 4,462,567; and 4,627,879, which are incorporated herein by reference. In general, fibrinogen and factor XIII are combined to form a first component that is mixed just prior to use with a second component containing thrombin. The thrombin converts the fibrinogen to fibrin, causing the mixture to gel, and activates the factor XIII. The activated factor XIII cross links the fibrin to strengthen and stabilize the adhesive matrix. Such adhesives typically contain from about 30 mg/ml to about 100 mg/ml fibrinogen and from about 50 µg/ml to about 500 µg/ml factor XIII. They may also contain additional ingredients, such as aprotinin, albumin, fibronectin, bulking agents, and solubilizers. Methods for producing factor XIII are known in the art. See, for example, U.S. Pat. No. 5,204,447. The fibrinogen is also useful for coating surfaces of polymeric articles, e.g. synthetic vascular grafts, as disclosed in U.S. Pat. No. 5,272,074 (incorporated herein by reference).

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example I

The multiple cloning site of the vector pUC18 (Yanisch-Perron et al., *Gene* 33:103-119, 1985) was removed and replaced with a synthetic double stranded oligonucleotide (the strands of which are shown in SEQ ID NO: 8 and SEQ ID NO: 27) containing the restriction sites Pvu I/Mlu I/Eco RV/Xba I/Pvu I/Mlu I, and flanked by 5' overhangs compatible with the restriction sites Eco RI and Hind III. pUC18 was cleaved with both Eco RI and Hind III, the 5' terminal phosphate groups were removed with calf intestinal phosphatase, and the oligonucleotide was ligated into the vector backbone. The DNA sequence across the junction was confirmed by sequencing, and the new plasmid was called pUCPM.

The β -lactoglobulin (BLG) gene sequences from pSS1tgXS (disclosed in WIPO publication WO 88/00239)

were excised as a Sal I-Xba I fragment and recloned into the vector pUCPM that had been cut with Sal I and Xba I to construct vector pUCXS. pUCXS is thus a pUC18 derivative containing the entire BLG gene from the Sal I site to the Xba I site of phage SS1 (Ali and Clark, *J. Mol. Biol.* 199: 415-426, 1988).

The plasmid pSS1tgSE (disclosed in WIPO publication WO 88/00239) contains a 1290 bp BLG fragment flanked by Sph I and EcoR I restriction sites, a region spanning a unique Not I site and a single Pvu II site which lies in the 5' untranslated leader of the BLG mRNA. Into this Pvu II site was ligated a double stranded, 8 bp DNA linker (5'-GGATATCC-3') encoding the recognition site for the enzyme Eco RV. This plasmid was called pSS1tgSE/RV. DNA sequences bounded by Sph I and Not I restriction sites in pSS1tgSE/RV were excised by enzymatic digestion and used to replace the equivalent fragment in pUCXS. The resulting plasmid was called pUCXSRV. The sequence of the BLG insert in pUCXSRV is shown in SEQ ID NO: 7, with the unique Eco RV site at nucleotide 4245 in the 5' untranslated leader region of the BLG gene. This site allows insertion of any additional DNA sequences under the control of the BLG promoter 3' to the transcription initiation site.

Using the primers BLGAMP3 (5'-TGG ATC CCC TGC CGG TGC CTC TGG-3'; SEQ ID NO: 9) and BLGAMP4 (5'-AAC GCG TCA TCC TCT GTG AGC CAG-3'; SEQ ID NO: 10) a PCR fragment of approximately 650 bp was produced from sequences immediately 3' to the stop codon of the BLG gene in pUCXSRV. The PCR fragment was engineered to have a BamH I site at its 5' end and an Mlu I site at its 3' end and was cloned as such into BamH I and Mlu I cut pGEM7zf(+) (Promega) to give pDAM200(+).

pUCXSRV was digested with Kpn I, and the largest, vector containing band was gel purified. This band contained the entire pUC plasmid sequences and some 3' non-coding sequences from the BLG gene. Into this backbone was ligated the small Kpn I fragment from pDAM200(+) which, in the correct orientation, effectively engineered a BamH I site at the extreme 5' end of the 2.6 Kbp of the BLG 3' flanking region. This plasmid was called pBLAC200. A 2.6 Kbp Cla I-Xba I fragment from pBLAC200 was ligated into Cla I-Xba I cut pSP72 vector (Promega), thus placing an EcoR V site immediately upstream of the BLG sequences. This plasmid was called pBLAC210.

The 2.6 Kbp Eco RV-Xba I fragment from pBLAC210 was ligated into Eco RV-Xba I cut pUCXSRV to form pMAD6. This, in effect, excised all coding and intron sequences from pUCXSRV, forming a BLG minigene consisting of 4.3 Kbp of 5' promoter and 2.6 Kbp of 3' downstream sequences flanking a unique EcoR V site. An oligonucleotide linker (ZC6839: ACTACGTAGT; SEQ ID NO: 11) was inserted into the Eco RV site of pMAD6. This modification destroyed the Eco RV site and created a Sna BI site to be used for cloning purposes. The vector was designated pMAD6-Sna. Messenger RNA initiates upstream of the Sna BI site and terminates downstream of the Sna BI site. The precursor transcript will encode a single BLG-derived intron, intron 6, which is entirely within the 3' untranslated region of the gene.

Example II

Clones encoding the individual fibrinogen chains were obtained from the laboratory of Dr. Earl W. Davie, University of Washington, Seattle. A genomic fibrinogen A α -chain clone (Chung et al., 1990, *ibid.*) was obtained from the plasmid BS4. This plasmid contains the A α clone inserted

into the Sal I and Bam HI sites of the vector pUC18, but lacks the coding sequence for the first four amino acids of the A α chain. A genomic B β -chain DNA (Chung et al., *ibid.*) was isolated from a lambda Charon 4A phage clone (designated $\beta\lambda 4$) as two EcoRI fragments of ca. 5.6 Kbp each. The two fragments were cloned separately into pUC19 that had been digested with Eco RI and treated with calf intestinal phosphatase. The resulting clones were screened by digestion with the restriction enzyme Pvu II to distinguish plasmids with the 5' and 3' B β inserts (designated Beta5'RI/puc and Beta3'RI/puc, respectively). Genomic γ -chain clones were isolated as described by Rixon et al. (*Biochemistry* 24: 2077-2086, 1985). Clone py12A9 comprises 5' non-coding sequences and approximately 4535 bp of γ -chain coding sequence. Clone py12F3 comprises the remaining coding sequence and 3' non-coding nucleotides. Both are pBR322-based plasmids with the fibrinogen sequences inserted at the EcoRI site. These plasmids were used as templates for the respective PCR reactions.

The fibrinogen chain coding sequences were tailored for insertion into expression vectors using the polymerase chain reaction (PCR) as generally described by Mullis (U.S. Pat. No. 4,683,202). This procedure removed native 5' and 3' untranslated sequences, added a 9 base sequence (CCT GCA GCC) upstream of the first ATG of each coding sequence, supplied the first four codons for the A α -chain sequence, removed an internal Mlu I site in the A α sequence and added restriction sites to facilitate subsequent cloning steps.

Referring to FIG. 1, the 5' end of the A α coding sequence was tailored in a PCR reaction containing 20 pmole for each of primers ZC6632 (SEQ ID NO: 12) and ZC6627 (SEQ ID NO: 13), approximately 10 ng of plasmid BS4 template DNA, 10 μ l of a mix containing 2.5 mM each dNTP, 7.5 μ l 10 \times *Pyrococcus furiosus* (Pfu) DNA polymerase buffer #1 (200 mM Tris-HCl, pH 8.2, 100 mM KCl, 60 mM (NH₄)₂SO₄, 20 mM MgCl₂, 1% Triton X-100, 100 μ g/ml nuclease free bovine serum albumin) (Stratagene, La Jolla, Calif.), and water to 75 μ l. The mixture was heated to 94 $^{\circ}$ C. in a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.). To the heated mixture was added 25 μ l of a mixture containing 2.5 μ l 10 \times Pfu buffer #1, 22 μ l H₂O and 1 μ l 2.5 units/ μ l Pfu DNA polymerase (Stratagene). The reactions were run in a DNA thermal cycler (Perkin-Elmer) for five cycles of 94 $^{\circ}$, 45 seconds; 40 $^{\circ}$, 90 seconds; 72 $^{\circ}$, 120 seconds; 20 cycles of 94 $^{\circ}$, 45 seconds; 45 $^{\circ}$, 90 seconds; 72 $^{\circ}$, 120 seconds; then incubated at 72 $^{\circ}$ for 7 minutes. The 5' PCR-generated fragment was digested with Bam HI and Hind III, and the Bam HI-Hind III fragment was then ligated to an internal 2.91 Kbp Hind III-Xba I fragment and Bam HI, Xba I-digested pUC18. PCR-generated exon sequences were sequenced.

Referring again to FIG. 1, the 3' end of the A α coding sequence was tailored in a series of steps in which the Mlu I site 563 bases upstream from the stop codon of the A α sequence was mutated using an overlap extension PCR reaction (Ho et al., *Gene* 77: 51-59, 1989). In the first reaction 40 pmole of each of primers ZC6521 (SEQ ID NO: 14) and ZC6520 (SEQ ID NO: 15) were combined with approximately 10 ng of plasmid BS4 template DNA in a reaction mixture as described above. The reaction was run for 5 cycles of 94 $^{\circ}$, 45 seconds; 40 $^{\circ}$, 60 seconds; 72 $^{\circ}$, 120 seconds; 15 cycles of 94 $^{\circ}$, 45 seconds; 45 $^{\circ}$, 60 seconds; 72 $^{\circ}$, 120 seconds; then incubated at 72 $^{\circ}$ for 7 minutes. A second reaction was carried out in the same manner using 40 pmole of each of primers ZC6519 (SEQ ID NO: 16) and ZC6518 (SEQ ID NO: 17) and BS4 as template. The PCR-generated DNA fragments from the first and second reactions were

isolated by gel electrophoresis and elution from the gel. Approximately 1/10 of each recovered reaction product was combined with 40 pmole of each of primers ZC6521 (SEQ ID NO: 14) and ZC6518 (SEQ ID NO: 17) in a PCR reaction in which the complementary 3' ends of each fragment (containing the single base change) annealed and served as a primer for the 3' extension of the complementary strand. PCR was carried out using the same reaction conditions as in the first and second 3' PCR steps. The reaction product was then digested with Xba I and Bam HI, and the Xba I-Bam HI fragment was cloned into Xba I, Bam HI-digested pUC18. PCR-generated exons were sequenced.

As shown in FIG. 1, the 5' Bam HI-Xba I fragment (3.9 Kbp) and the 3' Xba I-Bam HI fragment (1.3 Kbp) were inserted into the Bam HI site of the vector Zem228. Zem228 is a pUC18 derivative comprising a Bam HI cloning site between a mouse MT-1 promoter and SV40 terminator, and a neomycin resistance marker flanked by SV40 promoter and terminator sequences. See European Patent Office Publication EP 319,944 and FIG. 2. The entire α coding sequence was isolated from the Zem228 vector as an Sna BI fragment, which was inserted into the Sna BI site of the plasmid pMAD6-Sna.

Referring to FIG. 3, the 5' end of the B β -chain was tailored by PCR using the oligonucleotides ZC6629 (SEQ ID NO: 18), ZC6630 (SEQ ID NO: 19) and ZC6625 (SEQ ID NO: 20). These primers were used in pairwise combinations (ZC6629+ZC6625 or ZC6630+ZC6625) to generate B β coding sequences beginning at the first ATG codon (position 470 in SEQ ID NO: 3)(designated N1-Beta) or the third ATG codon (position 512 in SEQ ID NO: 3)(designated N3-Beta). Approximately 5 ng of Beta5'RI/puc template DNA was combined with 20 pmole of each of the primers (N1-Beta:ZC6629, SEQ ID NO: 18+ZC6625, SEQ ID NO: 20; or N3-Beta:ZC6630, SEQ ID NO: 19+ZC6625, SEQ ID NO: 20) in a reaction mixture as described above. The mixtures were incubated for 5 cycles of 94°, 45 seconds; 40°, 120 seconds; (N1-Beta) or 90 seconds (N3-Beta); 72°, 120 seconds; 20 cycles of 94°, 45 seconds; 45°, 120 seconds; (N1-Beta) or 90 seconds (N3-Beta); 72°, 120 seconds; then incubated at 72° for 7 minutes. The two reaction products N1, 555 bp or N3, 510 bp) were each digested with Eco RI and Bgl II, and the fragments were ligated to the internal Bgl II-Xba I fragment and Eco RI+Xba I-digested pUC19. The 3' end of the B β sequence was tailored in a reaction mixture as described above using the oligonucleotide primers ZC6626 (SEQ ID NO: 21) and ZC6624 (SEQ ID NO: 22) and approximately 5 ng of Beta3'RI/puc template. The mixtures were incubated for 5 cycles of 94°, 45 seconds; 40°, 90 seconds; 72°, 120 seconds; 15 cycles of 94°, 45 seconds; 45°, 90 seconds; 72°, 120 seconds; then incubated at 72° for 7 minutes. A 990 bp Bgl II-Eco RI fragment was isolated. This 3' fragment was ligated to the adjacent coding fragment (340 bp, SphI-Bgl II) and Sph I+Eco RI-digested pUC19. The 3' and 5' PCR-generated exons were sequenced. A third intermediate vector was constructed by combining two internal fragments (4285 bp Xba I-Eco RI and 383 kb Eco RI-Sph I) in Xba I+Sph I-digested pUC19. The entire B β coding sequence (two forms) was then assembled by ligating one of the 5' Eco RI-Xba I fragments, the internal Xba I-Sph I fragment, the 3' Sph I-Eco RI fragment and Eco RI-digested vector pUC19. The B β sequence was then isolated as a 7.6 Kbp Sna BI fragment and inserted into the Sna BI site of pMAD6-Sna.

Referring to FIG. 4, the 5' end of the gamma chain sequence was tailored by PCR using the oligonucleotide primers ZC6514 (SEQ ID NO: 23) and ZC6517 (SEQ ID

NO: 24) and approximately 50 ng of pY12A9 as template. The PCR reaction was run as described above using 40 pM of each primer. The reaction was run for 5 cycles of 94°, 45 seconds; 40°, 60 seconds, 72°, 120 seconds, followed by 15 cycles of 94°, 45 seconds; 45°, 60 seconds; 72°, 120 seconds. The resulting 213 bp fragment was digested with Bam HI and Spe I, and the resulting restriction fragment was ligated with the adjacent downstream 4.4 kb Spe I-Eco RI fragment and Bam HI+Eco RI digested pUC19. The 3' end of the gamma chain sequence was tailored using oligonucleotide primers ZC6516 (SEQ ID NO: 25) and ZC6515 (SEQ ID NO: 26) using 40 pM of each primer, approximately 50 ng of pY12F3 template and the same thermal cycling schedule as used for the 5' fragment. The resulting 500 bp fragment was digested with Spe I and Bam HI, and the resulting restriction fragment was ligated with the upstream 2.77 kb Eco RI-Spe I fragment and Eco RI+Bam HI-digested pUC19. All PCR-generated exons were sequenced. The entire γ -chain coding sequence was then assembled by ligating a 4.5 Kbp Bam HI-Eco RI 5' fragment, a 1.1 Kbp Eco RI-Pst I internal fragment and a 2.14 Kbp Pst I-Xba I 3' fragment in Bam HI+Xba I-digested Zem219b. Zem219b is a pUC18-derived vector containing a mouse metallothionein promoter and a DHFR selectable marker operably linked to an SV40 promoter (FIG. 5). Plasmid Zem219b has been deposited with American Type Culture Collection as an *E. coli* XL1-blue transformant under Accession No. 68979. The entire γ -chain coding sequence was then isolated as a 7.8 Kbp Sna B1 fragment and inserted into the Sna BI site of pMAD6-Sna.

Example III

Mice for initial breeding stocks (C57BL6J, CBACA) were obtained from Harlan Olac Ltd. (Bicester, UK). These were mated in pairs to produce F1 hybrid cross (B6CBAF1) for recipient female, superovulated females, stud males and vasectomized males. All animals were kept on a 14 hour light/10 hour dark cycle and fed water and food (Special Diet Services RM3, Edinburgh, Scotland) ad libitum.

Transgenic mice were generated essentially as described in Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1986, which is incorporated herein by reference in its entirety. Female B6CBAF1 animals were superovulated at 4-5 weeks of age by an i.p. injection of pregnant mares' serum gonadotrophin (FOLLIGON, Vet-Drug, Falkirk, Scotland) (5 iu) followed by an i.p. injection of human chorionic gonadotrophin (CHORULON, Vet-Drug, Falkirk, Scotland) (5 iu) 45 hours later. They were then mated with a stud male overnight. Such females were next examined for copulation plugs. Those that had mated were sacrificed, and their eggs were collected for microinjection.

DNA was injected into the fertilized eggs as described in Hogan et al. (ibid.) Briefly, each of the vectors containing the α , β and γ expression units was digested with Mlu I and the expression units were isolated by sucrose gradient centrifugation. All chemicals used were reagent grade (Sigma Chemical Co., St. Louis, Mo., U.S.A.), and all solutions were sterile and nuclease-free. Solutions of 20% and 40% sucrose in 1M NaCl, 20 mM Tris pH 8.0, 5 mM EDTA were prepared using UHP water and filter sterilized. A 30% sucrose solution was prepared by mixing equal volumes of the 20% and 40% solutions. A gradient was prepared by layering 0.5 ml steps of the 40%, 30% and 20% sucrose solutions into a 2 ml polyallomer tube and allowed to stand for one hour. 100 μ l of DNA solution (max. 8 μ g DNA) was loaded onto the top of the gradient, and the

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ence of all three protein chains at concentrations up to 1 mg/ml. The amount of fully assembled fibrinogen was related to the ratios of individual subunits present in the milk. No apparent phenotype was associated with high concentrations of human fibrinogen in mouse milk. 5

Example IV

Donor ewes are treated with an intravaginal progesterone-impregnated sponge (CHRONOGEST Goat Sponge, Intervet, Cambridge, UK) on day 0. Sponges are left in situ for ten or twelve days. 10

Superovulation is induced by treatment of donor ewes with a total of one unit of ovine follicle stimulating hormone (OFSH) (OVAGEN, Horizon Animal Reproduction Technology Pty. Ltd., New Zealand) administered in eight intramuscular injections of 0.125 units per injection starting at 5:00 pm on day -4 and ending at 8:00 am on day 0. Donors are injected intramuscularly with 0.5 ml of a luteolytic agent (ESTRUMATE, Vet-Drug) on day -4 to cause regression of the corpus luteum, to allow return to estrus and ovulation. To synchronize ovulation, the donor animals are injected intramuscularly with 2 ml of a synthetic releasing hormone analog (RECEPTAL, Vet-Drug) at 5:00 pm on day 0. 15 20

Donors are starved of food and water for at least 12 hours before artificial insemination (A.I.). The animals are artificially inseminated by intrauterine laparoscopy under sedation and local anesthesia on day 1. Either xylazine (ROMPUN, Vet-Drug) at a dose rate of 0.05–0.1 ml per 10 kg bodyweight or ACP injection 10 mg/ml (Vet-Drug) at a dose rate of 0.1 ml per 10 kg bodyweight is injected intramuscularly approximately fifteen minutes before A.I. to provide sedation. A.I. is carried out using freshly collected semen from a Poll Dorset ram. Semen is diluted with equal parts of filtered phosphate buffered saline, and 0.2 ml of the diluted semen is injected per uterine horn. Immediately pre- or post-A.I., donors are given an intramuscular injection of AMOXYPEN (Vet-Drug). 25 30 35

Fertilized eggs are recovered on day 2 following starvation of donors of food and water from 5:00 pm on day 1. Recovery is carried out under general anesthesia induced by an intravenous injection of 5% thiopentone sodium (INTRAVAL SODIUM, Vet-Drug) at a dose rate of 3 ml per 10 kg bodyweight. Anesthesia is maintained by inhalation of 1–2% Halothane/O₂/N₂O after intubation. To recover the fertilized eggs, a laparotomy incision is made, and the uterus is exteriorized. The eggs are recovered by retrograde flushing of the oviducts with Ovum Culture Medium (Advanced Protein Products, Brierly Hill, West Midlands, UK) supplemented with bovine serum albumin of New Zealand origin. After flushing, the uterus is returned to the abdomen, and the incision is closed. Donors are allowed to recover post-operatively or are euthanized. Donors that are allowed to recover are given an intramuscular injection of Amoxypen L.A. at the manufacturer's recommended dose rate immediately pre- or post-operatively. 40 45 50 55

Plasmids containing the three fibrinogen chain expression units are digested with Mlu I, and the expression unit fragments are recovered and purified on sucrose density gradients. The fragment concentrations are determined by fluorimetry and diluted in Dulbecco's phosphate buffered saline without calcium and magnesium as described above. The concentration is adjusted to 6 µg/ml and approximately 2 µl of the mixture is microinjected into one pronucleus of each fertilized eggs with visible pronuclei. 60 65

All fertilized eggs surviving pronuclear microinjection are cultured in vitro at 38.5° C. in an atmosphere of 5%

CO₂:5% O₂:90% N₂ and about -100% humidity in a bicarbonate buffered synthetic oviduct medium (see Table) supplemented with 20% v/v vasectomized ram serum. The serum may be heat inactivated at 56° C. for 30 minutes and stored frozen at -20° C. prior to use. The fertilized eggs are cultured for a suitable period of time to allow early embryo mortality (caused by the manipulation techniques) to occur. These dead or arrested embryos are discarded. Embryos having developed to 5 or 6 cell divisions are transferred to synchronized recipient ewes.

TABLE

Synthetic Oviduct Medium	
<u>Stock A (Lasts 3 Months)</u>	
NaCl	6.29 g
KCl	0.534 g
KH ₂ SO ₄	0.162 g
MgSO ₄ ·7H ₂ O	0.182 g
Penicillin	0.06 g
Sodium Lactate 60% syrup	0.6 mls
Super H ₂ O	99.4 mls
<u>Stock B (Lasts 2 weeks)</u>	
NaHCO ₃	0.21 g
Phenol red	0.001 g
Super H ₂ O	10 mls
<u>Stock C (Lasts 2 weeks)</u>	
Sodium Pyruvate	0.051 g
Super H ₂ O	10 mls
<u>Stock D (Lasts 3 months)</u>	
CaCl ₂ ·2H ₂ O	0.262 g
Super H ₂ O	10 mls
<u>Stock E (Lasts 3 months)</u>	
Hepes	0.651 g
Phenol red	0.001 g
Super H ₂ O	10 mls
To make up 10 mls of Bicarbonate Buffered Medium	
STOCK A	1 ml
STOCK B	1 ml
STOCK C	0.07 ml
STOCK D	0.1 ml
Super H ₂ O	7.83 ml
Osmolarity should be 265-285 mOsm. Add 2.5 ml of heat inactivated sheep serum and filter sterilize.	
<u>To make up 10 mls HEPES Buffered Medium</u>	
STOCK A	1 ml
STOCK B	0.2 ml
STOCK C	0.07 ml
STOCK D	0.1 ml
STOCK E	0.8 ml
Super H ₂ O	7.83 ml
Osmolarity should be 265-285 mOsm. Add 2.5 ml of heat inactivated sheep serum and filter sterilize.	

Recipient ewes are treated with an intravaginal progesterone-impregnated sponge (Chronogest Ewe Sponge or Chronogest Ewe-Lamb Sponge, Intervet) left in situ for 10 or 12 days. The ewes are injected intramuscularly with 1.5 ml (300 iu) of a follicle stimulating hormone substitute (P.M.S.G., Intervet) and with 0.5 ml of a luteolytic agent (Estrumate, Coopers Pitman-Moore) at sponge removal on day -1. The ewes are tested for estrus with a vasectomized ram between 8:00 am and 5:00 pm on days 0 and 1. Embryos surviving in vitro culture are returned to recipients (starved from 5:00 pm on day 5 or 6) on day 6 or 7. Embryo transfer is carried out under general anesthesia as

described above. The uterus is exteriorized via a laparotomy incision with or without laparoscopy. Embryos are returned to one or both uterine horns only in ewes with at least one suitable corpora lutea. After replacement of the uterus, the abdomen is closed, and the recipients are allowed to recover. 5 The animals are given an intramuscular injection of Amoxypen L.A. at the manufacturer's recommended dose rate immediately pre- or post-operatively.

Lambs are identified by ear tags and left with their dams for rearing. Ewes and lambs are either housed and fed 10 complete diet concentrates and other supplements and or ad lib. hay, or are let out to grass.

Within the first week of life (or as soon thereafter as possible without prejudicing health), each lamb is tested for the presence of the heterologous DNA by two sampling 15 procedures. A 10 ml blood sample is taken from the jugular vein into an EDTA vacutainer. If fit enough, the lambs also have a second 10 ml blood sample taken within one week of the first. Tissue samples are taken by tail biopsy as soon as possible after the tail has become desensitized after the 20 application of a rubber elastrator ring to its proximal third (usually within 200 minutes after "tailing"). The tissue is placed immediately in a solution of tail buffer. Tail samples are kept at room temperature and analyzed on the day of 25 collection. All lambs are given an intramuscular injection of Amoxypen L.A. at the manufacturer's recommended dose rate immediately post-biopsy, and the cut end of the tail is sprayed with an antibiotic spray.

DNA is extracted from sheep blood by first separating 30 white blood cells. A 10 ml sample of blood is diluted in 20 ml of Hank's buffered saline (HBS; obtained from Sigma

Chemical Co.). Ten ml of the diluted blood is layered over 5 ml of Histopaque (Sigma) in each of two 15 ml screw-capped tubes. The tubes are centrifuged at 3000 rpm (2000× g max.), low brake for 15 minutes at room temperature.

5 White cell interfaces are removed to a clean 15 ml tube and diluted to 15 ml in HBS. The diluted cells are spun at 3000 rpm for 10 minutes at room temperature, and the cell pellet is recovered and resuspended in 2–5 ml of tail buffer.

10 To extract DNA from the white cells, 10% SDS is added to the resuspended cells to a final concentration of 1%, and the tube is inverted to mix the solution. One mg of fresh proteinase K solution is added, and the mixture is incubated overnight at 45° C. DNA is extracted using an equal volume
15 of phenol/chloroform (×3) and chloroform/isoamyl alcohol (×1). The DNA is then precipitated by adding 0.1 volume of 3M NaOAc and 2 volumes of ethanol, and the tube is inverted to mix. The precipitated DNA is spooled out using a clean glass rod with a sealed end. The spool is washed in
20 70% ethanol, and the DNA is allowed to partially dry, then is redissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4).

DNA samples from blood and tail are analyzed by Southern blotting using probes for the BLG promoter region and
25 the fibrinogen chain coding regions.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described
30 herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 27

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5943 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(v i i) IMMEDIATE SOURCE:

(B) CLONE: Human Fibronogen A-alpha chain

(i x) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(31..84, 1154..1279, 1739..1922, 3055..3200, 3786..5210)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCTAGGAGC CAGCCCCACC CTTAGAAAAG ATG TTT TCC ATG AGG ATC GTC TGC	54
Met Phe Ser Met Arg Ile Val Cys	
1 5	
CTA GTT CTA AGT GTG GTG GGC ACA GCA TGG GTATGGCCCT TTTCATTTTT	104
Leu Val Leu Ser Val Val Gly Thr Ala Trp	
10 15	
TCTTCTTGCT TTCTCTCTGG TGTTTATTCC ACAAAGAGCC TGGAGGTCAG AGTCTACCTG	164
CTCTATGTCC TGACACACTC TTAGCTTTAT GACCCCAAGC CTGGGAGGAA ATTTCTCTGG	224

-continued

TGGGCTTGAC	ACCTCAAGAA	TACAGGGTAA	TATGACACCA	AGAGGAAAGAT	CTTAGATGGA		284
TGAGAGTGTA	CAACTACAA	GGAAGCTTTA	GCATCTGTCA	TTCAGTCTTA	CCACATTTTG		344
TTTTGTTTTG	TTTTAAAAAG	GGCAAGAATT	ATTTGCCATC	CTTGACCTA	TAAAGCCTTG		404
GTGCATTATA	ATGCTAGTTA	ATGGAATAAA	ACATTTTATG	GTAAGATTTG	TTTTCTTTAG		464
TTATTAATTT	CTTGCTACTT	GTCCATAATA	AGCAGAACTT	TTAGTGTTAG	TACAGTTTTG		524
CTGAAAAGTT	ATTGTTGTGT	TTGTCAAGAC	AGAAAGAAAA	GCAAAACGAAT	TATCTTTTGA		584
AATATCTTTG	CAGTATCAGA	AGAGATTAGT	TAGTAAGGCA	ATACGCTTTT	CCGCAGTAAAT		644
GGTATTCTTT	TAAATTATGA	ATCCATCTCT	AAAGGTTACA	TAGAAACTTG	AAAGGAGAGAG		704
GAACATTCA	TAAAGATAGT	CTAGGTTTTT	CTACTGAAAG	AGCAATTACA	GGAGAAAGAG		764
CTCTACAGTA	GTTTTCAACT	TTCTGTCTGC	AGTCATTAGT	AAAAATGAAA	AGGTAAAAAT		824
TAACTGATTT	TATAGATTCA	AATAATTTTC	CTTTTAGGAT	GGATTCTTTA	AAACTCTCTA		884
TATTTATCAA	ATGCTTATTT	AAGTGTACAC	CACAGTTAAG	AAATTTGTAC	ACCTTGTCTC		944
CTTTAATTCT	CATAACAACT	CCATAAAATG	GGTCCTAGGA	TTTCCATTTG	AAGATAAGAA		1004
ACCTGAAGCT	TGCCGAAGCC	CTGTGTCTGC	TCTCTTAAAT	CTCTGTGAGA	GTGCCATCTC		1064
TTCTGGGGGA	CTTGTAAGCA	TGCCACTGTC	TCCTCTTCTG	GCTAACATTG	CTGTTGCTCT		1124
CTTTTGTTTA	TGTGAATGAA	TCTTTAAAG	ACT GCA GAT	AGT GGT GAA	GGT GAC		1177
			Thr Ala Asp Ser Gly Glu Gly Asp				
			20		25		
TTT CTA GCT	GAA GGA GGA GGC GTG	CGT GGC CCA	AGG GTT GTG	GAA AGA			1225
Phe Leu Ala	Glu Gly Gly Gly Val	Arg Gly Pro	Arg Val Val	Glu Arg			
	30	35	40				
CAT CAA TCT	GCC TGC AAA GAT TCA	GAC TGG CCC	TTC TGC TCT	GAT GAA			1273
His Glu Ser	Ala Cys Lys Asp Ser	Asp Trp Pro	Phe Cys Ser	Asp Glu			
	45	50	55				
GAC TGG GTAAGCAGTC	AACGGGGGAA	GCAAGAGATT	CCTTCCCTCT	GATGCTAGAG			1329
Asp Trp							
60							
GGGCTCACAG	GCTGACCTGA	TTGGTCCCA	AAACTTTTTT	AAATAGAAAA	TAATTGAATA		1389
GTTACCTACA	TAGCAAAATA	AGAAAAAGAA	CCTACTCCCA	AGAGCACTGT	TTATTTACCT		1449
CCCCAACTCT	GGATCATTAG	TGGGTGAACA	GACAGGATTT	CAGTTGCATG	CTCAGGCAAA		1509
ACCAGGCTCC	TGAGTATTGT	GGCTTCAATT	TCCTGGCACC	TATTTATGGC	TAAGTGGACC		1569
CTCATTCAG	AGTTTCTCTG	CGACCTCTAA	CTAGTCTCT	TACCTACTTT	TAAGCCAACT		1629
TATCTGGAAG	AGAAAAAGGTA	GGAAAGAAATG	GGGGCTGCAT	GGAAACATGC	AAAATTATTG		1689
TGAATCTGAG	AGATAGATCC	TTACTGTAAAT	TTTCTCCCTT	CACTTTCAG	AAC TAC		1744
AAA TGC CCT	TCT GGC TGC	AGG ATG AAA	GGG TTG ATT	GAT GAA	GTC AAT		1792
Lys Cys Pro	Ser Gly Cys Arg Met	Lys Gly Leu	Ile Asp Glu	Val Asn			
	65	70	75				
CAA GAT TTT	ACA AAC AGA ATA	AAT AAG CTC	AAA AAT	TCA CTA	TTT GAA		1840
Glu Asp Phe	Thr Asn Arg Ile	Asn Lys Leu	Lys Asn	Ser Leu	Phe Glu		
	80	85	90				
TAT CAG AAG	AAC AAT AAG	GAT TCT CAT	TGG TTG ACC	ACT AAT	ATA ATG		1888
Tyr Glu Lys	Asn Asn Lys	Asp Ser His	Ser Leu	Thr Thr	Asn Ile Met		
	95	100	105				
GAA ATT TTG	AGA GGC	GAT TTT	TCC TCA	GCC AAT	A GTAAGTATTA		1932
Glu Ile Leu	Arg Gly Asp	Phe Ser	Ser Ala Asn				
	115		120				
CATATTTACT	TCTTTGACTT	TATAACAGAA	ACAACAAAAA	TCCTAAATAA	ATATGATATC		1992

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CGCTTATATC	TATGACAATT	TCATCCCAAA	GTACTTAGTG	TAGAAACACA	TACCTTCATA	2052
ATATCCCTGA	AAATTTTAA	AGGGAGCTTT	TGTTTTCGTT	ATTTTTTCAA	AGTAAAAAGAT	2112
GTAACTGAG	ATTGTTTAA	GTACAAAAAT	AAATCAGAA	TTTGGATTAA	AACAAAGAA	2172
TAAATGTGTT	CTTTTCAACA	GTATATACTG	AAAGTAGGAT	GGGTCAAGCT	CTTTGAGTTG	2232
ATATTTTTGT	TTCTGCTTTG	TAAAAGTGAA	AACTGAGAGG	TCAAAGGAAT	TGTTCAAAAG	2292
CACAGAGCTG	GGAAATTCAC	TCCCAGACTC	CACTGAGCTG	ATTAAGGTAGA	TTTTTAAAT	2352
TAAAAATATAG	GGTCAAGCTA	CGTCATTCTC	ACAGTCTACT	CATTAGGGTT	AGGAAACATT	2412
GCATTCACTC	TGGGCATGGA	CAGCGAATCT	AGGGAGTCC	CAGTTTCTCA	AGTTTGTCTT	2472
TGCCTTTTTA	CACCTTCACA	AACACTTGAC	ATTTAAATC	AGTGATGCCA	ACACTAGCTG	2532
GCAAGTGAGT	GATCCTGTTG	ACCCAAAAACA	GCTTAGGAAC	CATTTCAAA	CTATAGAGTT	2592
AAAAAGAAAA	GCTCATCAAT	AAGAAAAATCC	AATATGTTCA	AGTCCCTTGA	TAAAGATGT	2652
TATAAAATAA	TTGAAATGCA	ATCAAACCAA	CTATTTTAA	TCCAAATTAC	ACCTTTAAAA	2712
TTCCAAAGAA	AGTTCTTCTT	CTATATTTCT	TTGGGATTAC	TAATTGCTAT	TAGGACATCT	2772
TAACTGGCAT	TCATGGAAGG	CTGCAGGGCA	TAACATTATC	CAAAAAGTCAA	ATGCCCCATA	2832
GGTTTTGAAC	TCACAGATTA	AACGTGAACC	AAAAATAAAT	TAGGCATATT	TACAAAGCTAG	2892
TTTCTTTCTT	TCTTTTTTCT	CTTCTTTTCT	TTCTTTCTTT	CTTCTTTTCT	TTCTTTCTTT	2952
CTTCTTTTCT	TTCTCCTTCC	TTCTTTTCTT	CTTCTTTTCT	TTGCTGGCAA	TTACAGACAA	3012
ATCACTCAAG	AGCTACTTCA	ATAACCATAT	TTTCGATTTT	AG	AC COT GAT AAT	3063
Asn Arg Asp Asn 125						
ACC TAC AAC CGA GTG TCA GAG GAT CTG AGA AGC AGA ATT GAA GTC CTG	3113					
Thr Tyr Asn Arg Val Ser Glu Asp Leu Arg Ser Arg Ile Glu Val Leu						
130 135 140						
AAG CGC AAA GTC ATA GAA AAA GTA CAG CAT ATC CAG CTT CTG CAG AAA	3161					
Lys Arg Lys Val Ile Glu Lys Val Glu His Ile Glu Leu Leu Glu Lys						
145 150 155						
AAT GTT AGA GCT CAG TTG GTT GAT ATG AAA CGA CTG GAG GTAAATATGT	3210					
Asn Val Arg Ala Glu Leu Val Asp Met Lys Arg Leu Glu						
160 165 170						
GGCTGTGCTC CCGAGTGTC TTGTTTTTGA GTAGAGGGAA AAGGAAAGGC ATAGTTATGC	3270					
ACTGAGTGT TACTATATGC AGAGAAAAGT GTTATATCCA TCATCTACCT AAAAGTAAGT	3330					
ATTATTTTCC TCACTCCACA GTTGAAGAAA AAAAAATTCA GAGATATTAA GTAAATTTTC	3390					
CAACGTACAT AGATAGTAAT TCAAAGCAAT GTTCAGTCCC TGCTATTCC AAGCCATTAC	3450					
ATCACCACAC CTCTGAGCCC TCAGCCTGAG TTCACCAAGG ATCATTTAAT TAGCGTTTCC	3510					
TTTGAAGAGG AATAGCACCT TACTCTTGAT CCATTCTGAG GCTAAGATGA ATTAACAAGC	3570					
ATCCATTGCT TATCCTGGCT AGCCCTGCAA TACCCAACAT CTCTTCCACT GAGGGTGCTC	3630					
GATAGGCAGA AAACAGAGAA TATTAAGTGG TAGGTCTCCG AGTCAAAAAA AATGAAACCA	3690					
GTTCACAGAA GGAAATTTAA CTACCAAGAA CTCAATAAGC GTAGTTTATG TATTTGTATC	3750					
TACATTTTCT CTTTATTTTT CTCCCTCTC TCTAG GTG GAC ATT GAT ATT AAG	3803					
Val Asp Ile Asp Ile Lys 175						
ATC CGA TCT TGT CGA GGG TCA TGC AGT AGG GCT TTA GCT CGT GAA GTA	3851					
Ile Arg Ser Cys Arg Gly Ser Cys Ser Arg Ala Leu Ala Arg Glu Val						
180 185 190						
GAT CTG AAG GAC TAT GAA GAT CAG CAG AAG CAA CTT GAA CAG GTC ATT	3899					

00940 = 334260

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His	Pro	Asp	Glu	Ala	Ala	Phe	Phe	Asp	Thr	Ala	Ser	Thr	Gly	Lys	Thr	
		515					520					525				
TTC	CCA	GGT	TTC	TTC	TCA	CCT	ATG	TTA	GGA	GAG	TTT	GTC	AGT	GAG	ACT	4907
Phe	Pro	Gly	Phe	Phe	Ser	Pro	Met	Leu	Gly	Glu	Phe	Val	Ser	Glu	Thr	
	530					535					540					
GAG	TCT	AGG	GGC	TCA	GAA	TCT	GGC	ATC	TTC	ACA	AAT	ACA	AAG	GAA	TCC	4955
Glu	Ser	Arg	Gly	Ser	Glu	Ser	Gly	Ile	Phe	Thr	Asn	Thr	Lys	Glu	Ser	
	545				550				555					560		
AGT	TCT	CAT	CAC	CCT	GGG	ATA	GCT	GAA	TTC	CCT	TCC	CGT	GGT	AAA	TCT	5003
Ser	Ser	His	His	Pro	Gly	Ile	Ala	Glu	Phe	Pro	Ser	Arg	Gly	Lys	Ser	
				565				570						575		
TCA	AGT	TAC	AGC	AAA	CAA	TTT	ACT	AGT	AGC	ACG	AGT	TAC	AAC	AGA	GGA	5051
Ser	Ser	Tyr	Ser	Lys	Gln	Phe	Thr	Ser	Ser	Thr	Ser	Tyr	Asn	Arg	Gly	
				580				585					590			
GAC	TCC	ACA	TTT	GAA	AGC	AAG	AGC	TAT	AAA	ATG	GCA	GAT	GAG	GCC	GGA	5099
Asp	Ser	Thr	Phe	Glu	Ser	Lys	Ser	Tyr	Lys	Met	Ala	Asp	Glu	Ala	Gly	
	595						600					605				
AGT	GAA	GCC	GAT	CAT	GAA	GGA	ACA	CAT	AGC	ACC	AAG	AGA	GGC	CAT	GCT	5147
Ser	Glu	Ala	Asp	His	Glu	Gly	Thr	His	Ser	Thr	Lys	Arg	Gly	His	Ala	
	610					615					620					
AAA	TCT	CGC	CCT	GTC	AGA	GGT	ATC	CAC	ACT	TCT	CCT	TTG	GGG	AAG	CCT	5195
Lys	Ser	Arg	Pro	Val	Arg	Gly	Ile	His	Thr	Ser	Pro	Leu	Gly	Lys	Pro	
	625				630					635				640		
TCC	CTG	TCC	CCC	TAGACTAAGT	TAAATATTTT	TGCACAGTGT	TCCCATGGCC									5247
Ser	Leu	Ser	Pro													
				645												
CCTTGCAATTT	CCTTCTTAAC	TCTCTGTTAC	ACGTCATTGA	AACTACACTT	TTTTGGTCTG											5307
TTTTTGTGCT	AGACTGTAAG	TTCCTTGGGG	GCAGGGGCCTT	TGTCTGTCTC	ATCTCTGTAT											5367
TCCCAAATOC	CTAACAGTAC	AGAGCCATGA	CTCAATAAAT	ACATGTTAAA	TGGATGAATG											5427
AATTCTCTCTG	AAACTCTATT	TGAGCTTATT	TAGTCAAATT	CTTTCACTAT	TCAAAGTGTG											5487
TGCTATTAGA	ATTGTACACC	AACTGATTAA	TCACATTTTT	AGTATGTGTC	TCAATTOACA											5547
TTTAGGTCAO	GCTAAATACA	AGTTGTGTTA	GTATTAAAGTG	AGCTTAGCTA	CCTGTACTGG											5607
TTACTTGCTA	TTAGTTTGTG	CAAGTAAAAT	TCCAAATACA	TTTGAAGAAA	ATCCCCTTTTG											5667
CAATTTGTAG	GTATAAATAA	CCGCTTATTT	GCATAAGTTC	TATCCCACTG	TAAGTGCATC											5727
CTTTCCCTAT	GGAGGGAAAG	AAAGGAGGAA	GAAAGAAAGG	AAGGGAAAGA	AACAGTATTT											5787
GCCTTATTTA	ATCTGAGCCG	TGCCTATCTT	TGTAAAGTTA	AATGAGAATA	ACTTCTTCCA											5847
ACCAAGCTTAA	TTTTTTTTTT	AGACTGTGAT	GATGTCTCTC	AAACACATCC	TTCAGGTACC											5907
CAAAAGTGGA	TTTCAATAT	CAAGCTATCC	GGATCC													5943

(2) INFORMATION FOR SEQ ID NO2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 644 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO2:

Met Phe Ser Met Arg Ile Val Cys Leu Val Leu Ser Val Val Gly Thr
 1 5 10 15

Ala Trp Thr Ala Asp Ser Gly Glu Gly Asp Phe Leu Ala Glu Gly Gly
 20 25 30

Gly Val Arg Gly Pro Arg Val Val Glu Arg His Gln Ser Ala Cys Lys
 35 40 45

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Glu	Val	Val	Thr	Ser	Glu	Asp	Gly	Ser	Asp	Cys	Pro	Glu	Ala	Met	Asp
				485					490					495	
Leu	Gly	Thr	Leu	Ser	Gly	Ile	Gly	Thr	Leu	Asp	Gly	Phe	Arg	His	Arg
			500					505					510		
His	Pro	Asp	Glu	Ala	Ala	Phe	Phe	Asp	Thr	Ala	Ser	Thr	Gly	Lys	Thr
		515					520					525			
Phe	Pro	Gly	Phe	Phe	Ser	Pro	Met	Leu	Gly	Glu	Phe	Val	Ser	Glu	Thr
	530					535					540				
Glu	Ser	Arg	Gly	Ser	Glu	Ser	Gly	Ile	Phe	Thr	Asn	Thr	Lys	Glu	Ser
545					550				555					560	
Ser	Ser	His	His	Pro	Gly	Ile	Ala	Glu	Phe	Pro	Ser	Arg	Gly	Lys	Ser
				565					570					575	
Ser	Ser	Tyr	Ser	Lys	Gln	Phe	Thr	Ser	Ser	Thr	Ser	Tyr	Asn	Arg	Gly
			580					585					590		
Asp	Ser	Thr	Phe	Glu	Ser	Lys	Ser	Tyr	Lys	Met	Ala	Asp	Glu	Ala	Gly
		595					600					605			
Ser	Glu	Ala	Asp	His	Glu	Gly	Thr	His	Ser	Thr	Lys	Arg	Gly	His	Ala
	610					615					620				
Lys	Ser	Arg	Pro	Val	Arg	Gly	Ile	His	Thr	Ser	Pro	Leu	Gly	Lys	Pro
625					630					635				640	
Ser	Leu	Ser	Pro												

(2) INFORMATION FOR SEQ ID NO3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8878 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: human fibrinogen B-beta cDNA

(ix) FEATURE:

- (A) NAME/KEY: misc_RNA
- (B) LOCATION: 1..469

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 470..583

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 584..7257

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 7258..3449

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 3450..3938

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 3939..4122

(ix) FEATURE:

- (A) NAME/KEY: intron
- (B) LOCATION: 4123..5042

(ix) FEATURE:

- (A) NAME/KEY: exon
- (B) LOCATION: 5043..5270

(ix) FEATURE:

TTAATATAAG ATGTAACATA ATCATATTAT GTGCTTATTT TAATGAAATT AGCATTGCTT 683

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ATAGTTATGA	AATGGAATTG	TTAACCTCTG	ACTTATTGTA	TTTAAAGAAT	GTTTCATAGT	743
ATTTCTTATA	TAAAAACAAA	GTAATTTCTT	GTTTTCTAGT	TTATCACCTT	TGTTTTCTTA	803
AGATGAGGAT	GGCTTAAGTA	ATGTAAGATG	TGTTTTTCTC	ACTTGCTATT	CTGAGTACTG	863
TGATTTTCAT	TTACTTCTAG	CAATACAGGA	TTACAATTAA	GAAGACAAAG	TCTGAAAAATC	923
TCACAAACTA	TAAAAATAATA	AAAGAGCAGA	ATTTTAAAGT	AAAAGAAACT	GGTGOTAGGT	983
AGATTGTTC	TTGGTGAAAG	AAAGTAATAT	ATATTGTTAC	TGAGATTACT	ATTTATAAAA	1043
ATTATAACTA	AGCCTAAAAA	CAAAATACAT	CAAGTGTAAT	GATAGAAAAA	GAAATATTGC	1103
TTTTTTCAGA	TGAAAAAGTTC	AAATTAGAGT	TAGTGOTAT	TGTTATTATT	AATAGTTATG	1163
AAACACGGTT	CAGTCTAATT	TATTTATTTG	TAGAACAGTT	TGTCCCTCAAC	TATTATTTTT	1223
GCTGACTTAT	TGCTGTTAAT	TTGCAGTTAC	TAAAAATACA	GAAATGCATT	TAGGACAATG	1283
GATATTTAAG	AAATTTAAAT	TTTATCATCA	AACGTATCAT	GGCCAAATTT	CTTACATATA	1343
GATAGTATC	ATTAAACTAG	AAATAAGAAT	ACACAATAAT	ATTTAAATGA	AGTGATTTCAT	1403
TTGGGATCAT	TATTGAGTTT	CAAGGGAAC	TGAGTGTGTT	ACTTATCAGA	CTCTACATGT	1463
AAGAACATAT	AGTTAATCTG	GTTGTGTGTG	TAAAAACATA	TGGTTAATCT	GTTTAAAGTCT	1523
GGTTAATCAT	ATTAGGTAAAG	AAAAATGTAA	AGAATGTGTA	AGACGAAATT	TTTGTAAGGT	1583
ACTCTGCAAA	GCACCTTTCAC	ATTTCTGCTT	ATCAACTAAA	CCTCACAGAG	ATAGTTTAAAT	1643
AGTTTAGGCT	TTAAAAATGA	TTTTGATTAT	TCAACAAGTG	GCCTTCATAA	TTTCTTTAAG	1703
TGTTTTTCTT	TAAATATATA	CTTCTTTTAA	ATATTTTTTA	AAATTTCTTT	TTCTCTAGTA	1763
AAGCCAGACC	ATCCATGCTA	CCTCTCTAGT	GGCACTCTGA	AATAAAAAAG	AAATAGTTTT	1823
CTCTGTTATA	ATTGTATTTG	TAATAAGCAG	ATGAATCACA	TTTCTTAAAA	TTTGTTTTAG	1883
AGAGGGTAAAG	CTCTGACTAG	GACCATGACT	TCAATGTGAA	ATATGTATAT	ATCCTCCGAA	1943
TCCTTACATA	TTAAGAATGT	ATATAGTCAA	CTGGTTAAAC	AGGAAAAATCT	GGAACAGCCT	2003
GGCTGGGTTT	TAATCTTAAAG	ACCATCCTAC	TAAATGTTAA	ATAATATTAT	AATCTAATGA	2063
ATAAATGACA	ATGCAATTCC	AAATAGAGTT	CATCTGATGA	CTTCTAGACT	CACAAAAATTG	2123
CAAGAGAGCT	CAGTTGTTGC	TCAGTTGTTT	CAAAATCATGT	CGTTTGTAA	TTTGTAATTAA	2183
AGCTCCAAAG	GATGTATAAG	TACTGACAAA	AAAAAAAATG	AGAAATGTAGT	TAATCCAAAT	2243
CAAAACTTTT	CTATTGCAAT	GCATATTTT	TGCTTCATTA	TCCTTTAATA	TAATATTTTA	2303
AGTTAGCAAG	TAATTTTAAAT	TACAATGCAC	AAAGCCTTGAG	AATTATTTTA	AATATAAGAA	2363
AATCATAATG	TTTGATAAAAG	AAATCATGTA	AGAAATTTCA	AGATAATGGT	TTAACAAATA	2423
ATTTTGTTGA	TAGAAAGATAA	GACTAAAAAT	GAAATTCGAA	GTGGAGAGGA	CACTTAAACT	2483
GTAGTACTTG	TTATGTGTGA	TTCCAAGTAA	AATAATTAAT	AGCACTTATT	ATTGCCAAGT	2543
ACTGTTCTGA	GGGTACCATA	TGCAATAAAT	TATTTAATCC	TTACAATAAT	CTTGTAAGGC	2603
AGATTCAAAAC	TATCATTACA	CTTATTTTAC	AGATGAGAAA	ACTGGGGCAC	AGATAAAGCA	2663
ACTTGCCCAA	GGTCTCATAG	CTGTAAATCA	ACCCTACGGT	CAAGACCTAC	AAATAGCCGA	2723
GCTCCAGAGT	ACATTATGAG	GGTCAAAAGAT	TGTCTTATTA	CAAAATAAAT	CCAAATAGAA	2783
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ATTCACAAAA	ATGAAAGTAG	GGAAATGATT	AACAATAATC	ATAGGAATCT	AACAATCCAA	2903
GTGGCTTGAG	AATATTCATT	CTTCTTGACA	GTATAAGATT	TTTACAATTT	CGTAAAGTTCC	2963
AATGTATGTT	TTAGGAATAT	GAAGTCATTA	CTATTCATAA	TCTGATACAG	CTTTATCCTA	3023
AGGCCTCTCT	TTAAAAACTA	CACTGCATCA	TAGCTTTTTT	GTGCAGTTGG	TCTTTCTACT	3083

GTTACTGAAAC	AGTAAAGCAAC	CTACAGATTTC	ACTATCACCA	ACCAAGCCAGT	TGATGGATCT		3143
TAAGCAAATT	ATCAAAGCTTG	TGATAACCTA	AATTATAAAA	TGAAGGTGTT	GGAAATAGTTA		3203
CATTCCAAT	CTTCTATAAC	ACTCTGTATT	ATATTTCTGC	CTCATTCCTT	GTAG GGT		3260
					Gly		
TTC TTC AGT GCC CGT GGT CAT CGA CCC CTT GAC AAG AAG AGA GAA GAG							3308
Phe Phe Ser Ala Arg Gly His Arg Pro Leu Asp Lys Lys Arg Glu Glu							
40		45		50		55	
GCT CCC AGC CTG AGG CCT GCC CCA CCG CCC ATC AGT GGA GGT GGC TAT							3356
Ala Pro Ser Leu Arg Pro Ala Pro Pro Pro Ile Ser Gly Gly Gly Tyr							
		60		65		70	
CGG GCT CGT CCA GCC AAA GCA GCT GCC ACT CAA AAG AAA GTA GAA AGA							3404
Arg Ala Arg Pro Ala Lys Ala Ala Ala Thr Glu Lys Lys Val Glu Arg							
		75		80		85	
AAA GCC CCT GAT GCT GGA GGC TGT CTT CAC GCT GAC CCA GAC CTG							3449
Lys Ala Pro Asp Ala Gly Gly Cys Leu His Ala Asp Pro Asp Leu							
		90		95		100	
GTGGGTGCAC TGATGTTTTCT TGCAGTGGTG GCTCTCTCAT GCAGAGAAAG CCTGTATGTCA							3509
TGGCAGTCTG CTAATGTTTTC ACTGACCCAC ATTACCATCA CTGTTATTTT GTTTGTTTTAT							3569
TTTGGAAATA AAATTCAAAA CATAAACATA TTGGGCCCTTT GGTTTAGGCT TTCTTTCTTG							3629
TTTTCTTTGG TCTGGGCCCA AAATTTCAAA TTAGGATATG TGGGTGCCAC CTTTCCATTT							3689
GTATTTTGCC ACTGCCCTTG TTTAGTTGGT AAAATTTTCA TAGCCCAATT ATATTTTTTC							3749
TGGGGTAAAGT AATATTTTAA ATCTCTATGA GAGTATGATG ATGACTTTCG AATTTCTGGT							3809
CTTACAGAAA ACCAAATAAT AAATTTTTAT GTTGGCTAAT CGTATCGCTG AATTTTCCTA							3869
TGTGCTATTT TAACAAATGT CCATGACCCA AATCCTTCAT CTAATGCCTG CTATTTTCTT							3929
TOTTTTTAG GGG GTG TTG TGT CCT ACA GGA TGT CAG TTG CAA GAG GCT							3977
	Gly Val Leu Cys Pro Thr Gly Cys Glu Leu Glu Glu Ala						
		105		110		115	
TTG CTA CAA CAG GAA AGG CCA ATC AGA AAT AGT GTT GAT GAG TTA AAT							4025
Leu Leu Glu Glu Glu Arg Pro Ile Arg Asn Ser Val Asp Glu Leu Asn							
		120		125		130	
AAC AAT GTG GAA OCT GTT TCC CAG ACC TCC TCT TCT TCC TTT CAG TAC							4073
Asn Asn Val Glu Ala Val Ser Glu Thr Ser Ser Ser Ser Phe Glu Tyr							
		135		140		145	
ATG TAT TTG CTG AAA GAC CTG TGG CAA AAG AGG CAG AAG CAA GTA AAA G							4122
Met Tyr Leu Leu Lys Asp Leu Trp Glu Lys Arg Glu Lys Glu Val Lys							
		150		155		160	
GTAGATATCC TTGTGCTTTC CATTGCAATT TCAOCTATAA AATTGGAACC GTTAGACTGC							4182
CACGAGAATG CATGGTTGTG AGAAGATTAA CATTTCGTGG TTAGTGAATA GCATTCATAC							4242
GCITTTGGGC ACCTTCCCCT OCAACTTGCC AGATAAGCAC TATTCAGCTC TTATTTCCAG							4302
TCGACATCA GCAAGTGTGA TTTTCTATGA AAAATTCTAC TATGACTCCT TATTTTAAAGT							4362
ATACAAGAAA CTGTGACTC AGAAGATAAT ATTTACAGAG TGGAAAAAAA CCCCTAGCAT							4422
TTATAOTTIT AACATTTGAG OTTTTGAATG AGAGAGTTAT CCATAATATA TTCAATTGTG							4482
TTGTGGATAA TGACACCTAA CCTGTGAATC TTGAOGTCAG AATGTTGAGT GCTGTTGACT							4542
TGGTGGTCAG GAAACAGCTA GTGCGTGAGC CTGGCACAGG CATCTCAGTG AGTAGCATAC							4602
CCACAGTTGG AAATTTTTCA AAQAAATCAA AOGAATCATG ACATCTTATA AATTTCAAGG							4662
TTCTGCTATA CTTATGTGAA ATGGATAAAT AAATCAAQCA TATCCACTCT GTAAQATTGA							4722
ACTTCTCAGA TGGAAAGACC CAATACTGCT TTCTCCTCTT TTCCCTCACC AAAGAAATAA							4782
ACAACCTIAT TCATTTATTA CTGQACACAA TCTTTAGCGT ATACCTATGG TAAATTACTA							4842

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GTATGGTGGT	TAGGATTTAT	GTAAATTTGT	ATATGTCATG	CGCCAAATCA	TTTCCACTAA	4902
ATATGACTAT	ATATCATAAC	TGCTTGGTGA	TAGCTCAGTG	TTTAATAGTT	TATTCTCAGA	4962
AAATCAAAAT	TGTATAGTTA	AATACATTAG	TTTTATGAGG	CAAAAATGCT	AACTATTTCT	5022
ACATAATTTT	ATTTTTCAG	AT AAT GAA AAT GTA GTC AAT GAG TAC TCC				5071
		Asp Asa Glu Asa Val Val Asa Glu Tyr Ser				
		165			170	
TCA GAA CTG GAA AAG CAC CAA TTA TAT ATA GAT GAG ACT GTG AAT AGC						5119
Ser Glu Leu Glu Lys His Glu Leu Tyr Ile Asp Glu Thr Val Asa Ser						
	175		180		185	
AAT ATC CCA ACT AAC CTT CGT GTG CTT CGT TCA ATC CTG GAA AAC CTG						5167
Asa Ile Pro Thr Asa Leu Arg Val Leu Arg Ser Ile Leu Glu Asa Leu						
190		195		200		205
AGA AGC AAA ATA CAA AAG TTA GAA TCT GAT GTC TCA GCT CAA ATG GAA						5215
Arg Ser Lys Ile Glu Lys Leu Glu Ser Asp Val Ser Ala Glu Met Glu						
	210		215		220	
TAT TGT CGC ACC CCA TGC ACT GTC AGT TGC AAT ATT CCT GTG GTG TCT						5263
Tyr Cys Arg Thr Pro Cys Thr Val Ser Cys Asa Ile Pro Val Val Ser						
	225		230		235	
GGC AAA G GTAAGTATT CATAAACATA TTTTATAGAGA GTTCCAGAAAG AACTCACACA						5320
Gly Lys						
CCAAAAATAA GAGAACAAACA ACAACAACAA AAATGCTAAG TGGATTTTCC CAACAGATCA						5380
TAATGACATT ACAGTACATC ATAAAAATAT CCTTAGCCAG TTGTGTTTTG GACTGGCCTG						5440
GTGCATTTGC TGGTTTTGAT GAGCAGGATG GGGCACAGGT AGTCCCAAGG GTGGCTGATG						5500
TGTGCATCTG CGTACTGGCT TGAACAGATG GCAGAACCCAC AGATAGATGT AGAAGTTTCT						5560
CCATTTTGTG TGTCTGGGA GCTCATGGAT ATTCCAGGAC ACAAAAAGTG GAGAAAGAGCT						5620
TGTTTCATCC TCTTAGCAGA TAAACGTCTT CAAAAGTGGG TTGGACTTAC TAAAGTAAAA						5680
TGAAAAATCTA ATATTTGTTA TATTATTTTC AAAGGTCTAT AATAACACAC TCCTTAGTAA						5740
CTTATGTAAT GTTATTTTAA AGAATTGGTG ACTAAATACA AAGTAATTAT GTCATAAACC						5800
CCTGAACATA ATGTTGTCTT ACATTTGAG AA TGT GAG GAA ATT ATC AGG AAA						5853
			Glu Cys Glu Glu Ile Ile Arg Lys			
			240		245	
GGG GGT GAA ACA TCT GAA ATG TAT CTC ATT CAA CCT GAC AGT TCT GTC						5901
Gly Gly Glu Thr Ser Glu Met Tyr Leu Ile Glu Pro Asp Ser Ser Val						
	250		255		260	
AAA CCG TAT AGA GTA TAC TGT GAC ATG AAT ACA GAA AAT GGA G						5944
Lys Pro Tyr Arg Val Tyr Cys Asp Met Asa Thr Glu Asa Gly						
	265		270		275	
GTAAGCTTTT GACAGTTGTT GACCTGTTGA TCTGTAATTA TTTGGATACC GTAAAATGCC						6004
AGGAAACAAAG GCCAGGTGTG GTGGCTCATA CCTGTAATTTC CAGCACCTTG GGAAGGCCAAA						6064
GTGGGCTGAT AGCTTGAAGCC TAAGAGTTTG AAACTAAGCTT GGGCAACATA ATGAGACCCT						6124
AACTCTACAA AAAAAAAAAA AATACCAAAA AAAAAAAAAA AATCAGCTGT GTTGGTAGTA						6184
TGTGGCTGTA GTCCCAAGCTA TCCAGGAGGC TGAGATGGGA GATCACCTGA GCCCACAACC						6244
TGGAGTCTTG ATCATGCTAC TGAAGTGTAG CCTGGGCAAC AGAGGATAGT GAGATCCTGT						6304
CTCAAAAAAAAA AAAATTAATT AAAAAAGCCAG GAAACAAGAC TTAGCTCTAA CATCTAACAT						6364
AGCTGACAAA GGAAGTAATT GATGTGGAAT TCAACCTGAT ATTTAAAAAT TATAAAATAT						6424
CTATAATTCA CAATTTGGGG TAAGATAAAG CACTTGACGT TTCCAAAGAT TTTACAAGTT						6484
TACCTCTCAT ATTTATTTCC TTATTGTGTC TATTTTAGAG CACCAAATAT ATACTAAATG						6544
GAATGGACAG GGGATTGAGA TATTATTTTC AAAAGTACAT TATTTGCTGT TGGTTAATAT						6604

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ATGCTCTTTT TGTTCCTGTC AACCAAAAG GA TGG ACA GTG ATT CAG AAC CGT	6655
Gly Trp Thr Val Ile Glu Asn Arg 280 285	
CAA GAC GGT AGT GTT GAC TTT GGC AGG AAA TGG GAT CCA TAT AAA CAG	6703
Glu Asp Gly Ser Val Asp Phe Gly Arg Lys Trp Asp Pro Tyr Lys Glu 290 295 300	
GGG TTT GGA AAT GTT GCA ACC AAC ACA GAT GGG AAG AAT TAC TGT GGC	6751
Gly Phe Gly Asn Val Ala Thr Asn Thr Asp Gly Lys Asn Tyr Cys Gly 305 310 315	
CTA CCA G GTAACGAACA GGCATGCAAA ATAAAATCAT TCTATTTGAA ATGGGATTTT	6808
Leu Pro	
TTTAAATTAA AAAACATTCA TTGTTGGAAAG CCTGTTTTAG GCAGTTAAAG GAGATTTTCTT	6868
GACAAAAAATG TGGAAAGCTAA AGATAAAGGGA AGAAAAGGCAG TTTTATGTTT CCCAAAAATT	6928
TATTTTTGGT GAGAGATTTT ATTTTGTTTT TCTTTTATG GT GAA TAT TGG CTT	6980
Gly Glu Tyr Trp Leu 320	
GGG AAT GAT AAA ATT AGC CAG CTT ACC AGG ATG GGA CCC ACA GAA CTT	7028
Gly Asn Asp Lys Ile Ser Glu Leu Thr Arg Met Gly Pro Thr Glu Leu 325 330 335 340	
TTG ATA GAA ATG GAG GAC TGG AAA GGA GAC AAA GTA AAG GCT CAC TAT	7076
Leu Ile Glu Met Glu Asp Trp Lys Gly Asp Lys Val Lys Ala His Tyr 345 350 355	
GGG GGA TTC ACT GTA CAG AAT GAA GCC AAC AAA TAC CAG ATC TCA GTG	7124
Gly Gly Phe Thr Val Glu Asn Glu Ala Asn Lys Tyr Glu Ile Ser Val 360 365 370	
AAC AAA TAC AGA GGA ACA GCC GGT AAT GCC CTC ATG GAT GGA GCA TCT	7172
Asn Lys Tyr Arg Gly Thr Ala Gly Asn Ala Leu Met Asp Gly Ala Ser 375 380 385	
CAG CTG ATG GGA GAA AAC AAG ACC ATG ACC ATT CAC AAC GGC ATG TTC	7220
Glu Leu Met Gly Glu Asn Arg Thr Met Thr Ile His Asn Gly Met Phe 390 395 400	
TTC AGC ACG TAT GAC AGA GAC AAT GAC GGC TG GTATGTGTGG	7262
Phe Ser Thr Tyr Asp Arg Asp Asn Asp Gly Trp 405 410 415	
CACTCTTTTC TCCTGCTTTA AAAATCACAC TAATATCATT ACTCAGAATC ATTAACAATA	7322
TTTTTAATAG CTACCACTTC CTGGGCACTT ACTGTCAGCC ACTGTCTTAA GCTCTTTATG	7382
CATCACTCGA AAGCATTTC AACTATAAGT AGACATTCTT ATTCTCATTT TACAGATGAG	7442
ATTTAAGAGG ATTACGTGAT TTGTCCAATG TCACACAACCT ACCCAGAGAT AAAACTAGAA	7502
TTTGAGCACA GTTACTTTCT GAATAATGAG CATTTAAGATA AATACCTATA TCTCTATATT	7562
CTAAAGTGTTG TGTGAAAACT TTCATTTTCA TTTCAGGGT TCTCTGATAC TAAGGGTTGT	7622
AAAAAGCTATT ATTCCAGTAT AAAGTAACAA ACACAATCCC TAGATGGATT GCCACAAAAG	7682
CCCAAGTTATC TCTCTTTCTT GCTATAAGGC ACAGGAGGTC TTTGGTGTAT TAGTGTGACT	7742
CTATGTATAG CACCCAAAAGG AAAGACTACT GTGCACACGA GTGTAGCAGT CTTTATGGG	7802
TAATCTGCAA AACGTAACTT GACCACCGTA GTTCTGTTTC TAATAACGCC AAACACATTT	7862
TCTTTTCA G TTA ACA TCA GAT CCC AGA AAA CAG TGT TCT AAA GAA GAC	7910
Leu Thr Ser Asp Pro Arg Lys Glu Cys Ser Lys Glu Asp 420 425	
GGT GGT GGA TGG TGG TAT AAT AGA TGT CAT GCA GCC AAT CCA AAC GGC	7958
Gly Gly Gly Trp Trp Tyr Asn Arg Cys His Ala Ala Asn Pro Asn Gly 430 435 440	
AGA TAC TAC TGG GGT GGA CAG TAC ACC TGG GAC ATG GCA AAG CAT GGC	8006
Arg Tyr Tyr Trp Gly Gly Glu Tyr Thr Trp Asp Met Ala Lys His Gly	

6655-7958

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445	450	455	460	
ACA GAT GAT GGT GTA GTA TGG ATG AAT TGG AAG GGG TCA TGG TAC TCA				8054
Thr Asp Asp Gly Val Val Trp Met Asa Trp Lys Gly Ser Trp Tyr Ser				
	465	470	475	
ATG AGG AAG ATG AGT ATG AAG ATC AGG CCC TTC TTC CCA CAG CAA TAGTCCCC				8109
Met Arg Lys Met Ser Met Lys Ile Arg Pro Phe Phe Pro Glu Glu				
	480	485	490	
TACGTAGATT TTTGCTCTTC TGTATGTGAC AACATTTTTG TACATTATGT TATTGGAATT				8169
TTCTTTCATA CATTATATT CTTCTAAACT CTCAAGCAGA CGTGAGTGTG ACTTTTTGAA				8229
AAAAGTATAG GATAAATTAC ATTAAAAATAG CACATGATTT TCTTTTGITT TCTTCATTTT				8289
TCTTGCTCAC CCAAGAAAGTA ACAAAGTAT AGTTTTGACA GAGTTGGTGT TCATAATTTT				8349
AGTTCTAGTT GATTGCGAGA ATTTTCAAAT AAGGAAGAGG GGTCITTTTAT CCTTGTCGTA				8409
GGAAAACCAT GACGGAAAAGG AAAAACTGAT GTTTAAAAAGT CCACTTTTAA AACTATATTT				8469
ATTTATGTAG GATCTGTCAA AGAAAACITC CAAAAAGATT TATTAATTAA ACCAGACTCT				8529
OTTGCAATAA OTTAATGTTT TCTTGTTTTG TAATCCACAC ATTCAATGAG TTAGGCTTTG				8589
CACTTGTAAG GAAAGGAGAA GCTTCACAAC CTCAAATAGC TAATAAACCG GTCTTGAATA				8649
TTTGAAGATT TAAAACTCTGA CTCTAGGACG GGCACGGTGG CTCACGACTA TAATCCCAAC				8709
ACTTTGGGAG GCTGAGGCGG GCGGTCACAA GGTGAGGAGT TCAAGACCAG CCTGACCAAT				8769
ATGGTGAAAC CCCATCTCTA CTAAAAATAC AAAAAATTAGC CAGGCGTGGT GGCAGGTGCC				8829
TGTAGGTCCC AGCTAGCCTG TGAGGTGGAG ATTGCATTGA GCCAAGATC				8878

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 491 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Lys	Arg	Met	Val	Ser	Trp	Ser	Phe	His	Lys	Leu	Lys	Thr	Met	Lys	
1				5					10					15		
His	Leu	Leu	Leu	Leu	Leu	Leu	Cys	Val	Phe	Leu	Val	Lys	Ser	Glu	Gly	
			20					25					30			
Val	Asn	Asp	Asn	Glu	Glu	Gly	Phe	Phe	Ser	Ala	Arg	Gly	His	Arg	Pro	
		35					40					45				
Leu	Asp	Lys	Lys	Arg	Glu	Glu	Ala	Pro	Ser	Leu	Arg	Pro	Ala	Pro	Pro	
	50					55					60					
Pro	Ile	Ser	Gly	Gly	Gly	Tyr	Arg	Ala	Arg	Pro	Ala	Lys	Ala	Ala	Ala	
	65			70					75					80		
Thr	Glu	Lys	Lys	Val	Glu	Arg	Lys	Ala	Pro	Asp	Ala	Gly	Gly	Cys	Leu	
			85					90					95			
His	Ala	Asp	Pro	Asp	Leu	Gly	Val	Leu	Cys	Pro	Thr	Gly	Cys	Glu	Leu	
		100					105					110				
Glu	Glu	Ala	Leu	Leu	Glu	Glu	Glu	Arg	Pro	Ile	Arg	Asn	Ser	Val	Asp	
		115				120					125					
Glu	Leu	Asn	Asn	Asn	Val	Glu	Ala	Val	Ser	Glu	Thr	Ser	Ser	Ser	Ser	
	130					135					140					
Phe	Glu	Tyr	Met	Tyr	Leu	Leu	Lys	Asp	Leu	Trp	Glu	Lys	Arg	Glu	Lys	
	145				150				155					160		
Glu	Val	Lys	Asp	Asn	Glu	Asn	Val	Val	Asn	Glu	Tyr	Ser	Ser	Glu	Leu	
			165					170						175		

009496 = 04450

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Glu	Lys	His	Gln	Leu	Tyr	Ile	Asp	Glu	Thr	Val	Asn	Ser	Asn	Ile	Pro	180	185	190
Thr	Asn	Leu	Arg	Val	Leu	Arg	Ser	Ile	Leu	Glu	Asn	Leu	Arg	Ser	Lys	195	200	205
Ile	Gln	Lys	Leu	Glu	Ser	Asp	Val	Ser	Ala	Gln	Met	Glu	Tyr	Cys	Arg	210	215	220
Thr	Pro	Cys	Thr	Val	Ser	Cys	Asn	Ile	Pro	Val	Val	Ser	Gly	Lys	Glu	225	230	235
Cys	Glu	Glu	Ile	Ile	Arg	Lys	Gly	Gly	Glu	Thr	Ser	Glu	Met	Tyr	Leu	245	250	255
Ile	Gln	Pro	Asp	Ser	Ser	Val	Lys	Pro	Tyr	Arg	Val	Tyr	Cys	Asp	Met	260	265	270
Asn	Thr	Glu	Asn	Gly	Gly	Trp	Thr	Val	Ile	Gln	Asn	Arg	Gln	Asp	Gly	275	280	285
Ser	Val	Asp	Phe	Gly	Arg	Lys	Trp	Asp	Pro	Tyr	Lys	Gln	Gly	Phe	Gly	290	295	300
Asn	Val	Ala	Thr	Asn	Thr	Asp	Gly	Lys	Asn	Tyr	Cys	Gly	Leu	Pro	Gly	305	310	315
Glu	Tyr	Trp	Leu	Gly	Asn	Asp	Lys	Ile	Ser	Gln	Leu	Thr	Arg	Met	Gly	325	330	335
Pro	Thr	Glu	Leu	Leu	Ile	Glu	Met	Glu	Asp	Trp	Lys	Gly	Asp	Lys	Val	340	345	350
Lys	Ala	His	Tyr	Gly	Gly	Phe	Thr	Val	Gln	Asn	Glu	Ala	Asn	Lys	Tyr	355	360	365
Gln	Ile	Ser	Val	Asn	Lys	Tyr	Arg	Gly	Thr	Ala	Gly	Asn	Ala	Leu	Met	370	375	380
Asp	Gly	Ala	Ser	Gln	Leu	Met	Gly	Glu	Asn	Arg	Thr	Met	Thr	Ile	His	385	390	395
Asn	Gly	Met	Phe	Phe	Ser	Thr	Tyr	Asp	Arg	Asp	Asn	Asp	Gly	Trp	Leu	405	410	415
Thr	Ser	Asp	Pro	Arg	Lys	Gln	Cys	Ser	Lys	Glu	Asp	Gly	Gly	Gly	Trp	420	425	430
Trp	Tyr	Asn	Arg	Cys	His	Ala	Ala	Asn	Pro	Asn	Gly	Arg	Tyr	Tyr	Trp	435	440	445
Gly	Gly	Gln	Tyr	Thr	Trp	Asp	Met	Ala	Lys	His	Gly	Thr	Asp	Asp	Gly	450	455	460
Val	Val	Trp	Met	Asn	Trp	Lys	Gly	Ser	Trp	Tyr	Ser	Met	Arg	Lys	Met	465	470	475
Ser	Met	Lys	Ile	Arg	Pro	Phe	Phe	Pro	Gln	Gln						485	490	

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10564 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: human fibrinogen gamma chain

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: join(1799..1876, 1973..2017, 2207..2390, 2510
... 2603, 4211..4341, 4645..4778, 5758..5942, 7426
... 7703, 9342..9571)

669400 669400 669400

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTACACACTT	CTTGAAAGCA	AAGGCAATGC	TGAAGTCACC	TTTCATGTTT	AAATCATATT	60
AAAAAGTTAG	CAAGATGTAA	TTATCAGTGT	ACTATGTAAA	TCTTTGTGAA	TGATCAATAA	120
TTACATATTT	TCATTATATA	TATTTTAGTA	GATAATATTT	ATATACATTC	AACATTCTAA	180
ATATAGAAAAG	TTTACAGAGA	AAAAATAAAGC	CTTTTTTTTCC	AATCCTGTCC	TCCACCTCTG	240
CATCCCATTG	TTCTTCACAG	AAGCAACTGA	TTCAAGTCAT	TACATAGTTA	TTGAGTGTTA	300
ACTACAACATA	TGTTAAAGTAC	AGCTATATAT	GTTAGATGCC	GTAGCCACAG	AAATCAGTTT	360
ACAATCTAAT	GCAAGTGGATA	CAGCATGTAT	ACATATAATA	TAAGGTTTGT	ACAAATGCTA	420
TCTGAGGTAG	AGCTGTTTGA	AAGAATACTA	ATACTTAAAT	GTTTAATTCA	ACTGACTTGA	480
TTGACAACCTG	ATTAGCTGAG	TGGAAAAAGAT	GGATGAGAAA	GATTGTGAGA	CTTAATTGGC	540
TGGTGGTATG	GTGATATGAT	TGACAATAAC	TGCTAAGTCA	GAGAGGGATA	TATTAAGGAG	600
GAGAAAGAAA	GCAACAAATC	TGGTTTTGAT	GTGTTCACTT	TGTTATAATT	ATTGATTATT	660
TACTGAATAT	GAATATTTAT	CTTTGTTTTT	GAGTCAATAA	ATATACCTTT	GTAAAGACAG	720
AATTAAGTA	TTAGTATTTT	TTTCAAACCTG	GAGGCATTTT	TCCCACTAAC	ATATTTTCATC	780
AAAACTTATA	ATAAGCTTGG	TTCCAAGAGGA	AGAAATGAGG	GATAACCCAA	AATAGAGACA	840
TTAATAATAG	TGTAACGCCC	AGTGATAAAT	CTCAATAAGC	AGTGATGACA	GACATGTTTT	900
CCCAAACACA	AGGATGCTGT	AAGGGCCAAA	CAGAAATGAT	GGCCCTCCC	CAGCACCTCA	960
TTTTGCCCTT	TCCTTCAOCT	ATGCCCTCTAC	TCTCCTTTAG	ATACAAGGGA	GGTGGATTTT	1020
TCTCTTCTCT	GAGATAGCTT	GATGGAACCA	CAGGAACAAT	GAAAGTGGGT	CCTGGCTCTT	1080
TTCTCTGTGG	CAGATGGGGT	GCCATGCCCA	CCTTCAGACA	AAAGGAAAGAT	TGAGCTCAAA	1140
AGCTCCCTGA	GAAAGTGAGG	CCTATGAACA	TGTTTGACAC	AGAGGGACAG	GAATGTATTT	1200
CCAGGGTCAT	TCATTCCCTGG	GAATAAGTAA	CTGGGACATG	GGGGAAAGTCA	GTCTCCTCCT	1260
GCCACAGCCA	CAGATTAAAA	ATAATAATGT	TAAGTGATCC	CTAGGCTAAA	ATAATAGTGT	1320
TAAGTGATCC	CTAAGCTAAG	AAAGTTCTTT	TGGTAATTCA	GGTGATGGCA	GCAGGACCCA	1380
TCTTAAGGAT	AGACTAGGTT	TGCTTAAGTT	GAGGTTCATAT	CTGTTTGCTC	TCAGCCATGT	1440
ACTGGAAGAA	GTTGCATCAC	ACAGCCTCCA	GGACTGCCCT	CCTCCTCACA	GCAATGGATA	1500
ATGCTTCACT	AGCCTTTGCA	GATAATTTTG	GATCAGAGAA	AAAACCTTGA	GCTGGGCCAA	1560
AAAGGAGGAG	CTTCAACCTG	TGTGCAAAAT	CTGGGAACCT	GACAGTATAG	GTTGGGGGCC	1620
AGGATGAGGA	AAAAAGGAACG	GGAAAGACCT	GGCCACCCTT	CTGGTAAGGA	GGCCCCGTGA	1680
TCAGCTCCAAG	CCATTTGCAAG	TCCTGGCTAT	CCCAAGAGCT	TACATAAAGG	GACAATTGGA	1740
GCCTGAGAGG	TGACAGTGCT	GACACTACAA	GGCTCGGAGC	TCCGGGCACT	CAGACATC	1798
ATG AGT TGG TCC TTG CAC CCC CGG AAT TTA ATT CTC TAC TTC TAT GCT	1846					
Met Ser Trp Ser Leu His Pro Arg Asa Leu Ile Leu Tyr Phe Tyr Ala						
1 5 10 15						
CTT TTA TTT CTC TCT TCA ACA TGT GTA GCA GTAAAGTGTGC TCTTCACAAA	1896					
Leu Leu Phe Leu Ser Ser Thr Cys Val Ala						
20 25						
ACGTTGTTTA AAATGGAAAAG CTGGAAAATA AAACAGATAA TAAACTAGTG AAATTTTCGT	1956					
ATTTTTTCTC TTTTAG TAT GTT GCT ACC AGA GAC AAC TGC TGC ATC TTA	2005					
Tyr Val Ala Thr Arg Asp Asa Cys Cys Ile Leu						
30 35						
GAT GAA AGA TTC GTAAGTAGTT TTTATGTTTC TCCCTTTGTG TGTGAACGG	2057					
Asp Glu Arg Phe						

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40										
AGAGGGGCGAG	AGGAATAGAA	ATAATTCCT	CATAAATATC	ATCTGGCACT	TGTAACTTTT					2117
TAAAAACATA	GTCTAGGTTT	TACCTATTTT	TCTTAATAGA	TTTTAAGAGT	AGCATCTGTC					2177
TACATTTTITA	ATCACTGTTA	TATTTTCAG	GGT AGT TAT	TGT CCA ACT	ACC TGT					2230
Gly Ser Tyr Cys Pro Thr Thr Cys										
45										
GQC ATT GCA GAT TTC CTG TCT ACT TAT CAA ACC	AAA GTA GAC AAG GAT									2278
Gly Ile Ala Asp Phe	Leu Ser Thr Tyr Glu Thr									
50	55			60						65
CTA CAG TCT TTG GAA GAC ATC TTA CAT CAA GTT	GAA AAC AAA ACA TCA									2326
Leu Glu Ser Leu Glu Asp Ile Leu His Glu Val	Glu Asn Lys Thr Ser									
70	75									80
GAA GTC AAA CAG CTG ATA AAA GCA ATC CAA CTC	ACT TAT AAT CCT GAT									2374
Glu Val Lys Glu Leu Ile Lys Ala	Ile Glu Leu Thr Tyr Asn Pro Asp									
85	90									95
GAA TCA TCA AAA CCA	A GTGAGAAAAT AAAGACTACT GACCAAAAAA									2420
Glu Ser Ser Lys Pro										
100										
TAATAATAAT AATCTGTGAA GTTCTTTTGC	TGTTGTTTTA GTTGTTCCTAT TTGCTTAAGG									2480
ATTTTTATGT CTCTGATCCT ATATTACAG	AT ATG ATA GAC GCT GCT ACT TTG									2532
Asn Met Ile Asp Ala Ala Thr Leu										
105										110
AAG TCC AGG ATA ATG TTA GAA GAA ATT ATG AAA	TAT GAA GCA TCG ATT									2580
Lys Ser Arg Ile Met Leu Glu Glu	Ile Met Lys Tyr Glu Ala Ser Ile									
115	120									125
TTA ACA CAT GAC TCA AGT ATT CG	GTAAGGATTT TTGTTTTAAT TTGCTCTGCA									2633
Leu Thr His Asp Ser Ser Ile Arg										
130										
AGACTGATTT AGTTTTTATT TAATATTCTA	TACTTGAGTG AAAGTAATTT TTAATGTGTT									2693
TTCCCCATTT ATAATATCCC AGTGACATTA	TGCTTGATTA TGTTOAGCAT AGTAGAGATA									2753
GAAATTTTTA GTGCAATATA AATTATACTG	GGTTATAAAT GCTTATTAAT AATCACATTG									2813
AAGAAAGATG TTCTAGATGT CTTCAAATGC	TAOTTTGACC ATATTTATCA AAAATTTTTT									2873
CCCCATCCCC CATTTATCTT ACAACATAAA	ATCAATCTCA TAGGAATTTG GGTOTTGAAA									2933
ATAAAATCCT CTTTATAAAA ATGCTGACAA	ATTGGTGGTT AAAAAAATTA GCAAGCAGAG									2993
GCATAGTAAAG GATTTTGGCT CCTAAAGTAA	ATTATATTGA ATGTGGAGCA GGAAGAAACA									3053
TGTCTTGAGA GACTAAGTGT GC AAAATATT	GCAAGCTCA TATTGATCAT TGCAAGATGA									3113
ACCTGCATAG TCTCTTCCCT TCATTTGGAA	GTGAATGTCT CTGTTAAAAGC TTCTCAGGGA									3173
CTCATAAACT TTCTGAACAT AAGGTCTCAG	ATACAGTTTT AATATTTTTT CCAATTTTTT									3233
TTTTCTGAAT TTTTCTCAAA GCAGCTTGAG	AAATTOAGAT AAATAGTAGC TAGGGAGAAAG									3293
TGCCCCAGGA AAGATTTCTC CTCTTTTTGC	TATCAAGAGG CCCTTOTTAT TATTGTTATT									3353
ATTATTACTT GCATTATTAT TGTCCATCAT	TGAATTTGAA GGAGGTTATT GTACAGAAAT									3413
TGCCTAAGAC AAGGTAGAGG GAAAAAGTGG	ACAAATAGTT TGTCTACCCT TTTTACTTC									3473
AAAGAAAGAA CGGTTTATGC ATTGTAGACA	GTTTCTATC ATTTTGGAT ATTTGCAAGC									3533
CACCTGTAA GTAACACAA AAGGAAGGTT	TTTACTTCCC CCAGTCCATT CCCAAAGCTA									3593
TGTAACCAGA AGCATTAAAA AAGAAAAGGG	AAGTATCTGT TGTTTTATTT TACATACAA									3653
AACGTTCCAG ATCATGTCCC TGTOTAAOTT	ATATTTTGA TTAAGCTTA TATGTATAAGC									3713
CTCAGTAGAT CCACAAGTGA AAGGTATACT	CCTTCAGCAC ATGTGAATTA CTGAAGTGA									3773

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CTTTTCCTGC	TTCTAAAGCA	TCAGGGGGTG	TTCCTATTAA	CCAGTCTCOC	CAC TCTTCCA	3893
GGTTGCTATC	TGCTGTCCCT	TATGCATAAA	GTAAAAAGCA	AAATGTCAAT	GACATTTGCT	3893
TATTGACAAO	GACTTTGTTA	TTTGTGTTGG	GAGTTGAGAC	AAATATCCCC	ATTCTAAOTA	3953
AAAAGATTCA	GGTCCACATT	GTATTCCTGT	TTTAATTGAT	TTTTTGATTT	GT TTTTCTTT	4013
TTCAAAAAOT	TTATAATTTT	AATTCATGTT	AATTTAGTAA	TATAATTTTA	CATTTTCCTC	4073
AAGAATGAAA	TAATTTATCA	GAAAOCACIT	CTTAAGAAAA	TACTTAGCAG	TTTCCAAAQA	4133
AAATATAAAA	TTACTCTTCT	GAAAOGAATA	CTTATTTITG	TCTTCTTATT	TTTOTTATCT	4193
TATGTTTCTG	TTTGTAG A	TAT TTG CAG	GAA ATA TAT	AAT TCA AAT	AAT CAA	4244
		Tyr Leu Glu Glu Ile Tyr Asn Ser Asn Asn Glu				
		135		140		145
AAG ATT GTT AAC CTG AAA GAG AAG GTA GCC CAG CTT GAA GCA CAG TGC						4292
Lys Ile Val Asn Leu Lys Glu Lys Val Ala Glu Leu Glu Ala Glu Cys						
	150		155		160	
CAG GAA CCT TGC AAA GAC ACG GTG CAA ATC CAT GAT ATC ACT GGG AAA O						4341
Glu Glu Pro Cys Lys Asp Thr Val Glu Ile His Asp Ile Thr Gly Lys						
	165		170		175	
GTAAGTGATG AAGGTTATAT TGGGATTAGG TTICATCAAG TAAGTAATGT AAAGGAGAAA						4401
GTATGTACTG GAAAGTATAG GAATAGTTTA GAAAGTGCGT ACCCATTAAG TCTAAGAAAT						4461
TCAGTTGTCT AGACCTTTCT TGAATAAGCTA AAAAAAACAG TTTAAAAGGA ATGCTGATGT						4521
GAAAAGTAAO AAAATTATTC TTGAAAAATG AATAGTTTAC TACATGTTAA AAGCTATTTT						4581
TCAAGGCTGO CACAGTCTTA CCTGCATTTT AAACCACAGT AAAAGTCGAT TCTCCTTCTC						4641
TAG AT TGT CAA GAC ATT GCC AAT AAG GGA GCT AAA CAG AGC GGG CTT						4688
Asp Cys Glu Asp Ile Ala Asn Lys Gly Ala Lys Glu Ser Gly Leu						
	180		185		190	
TAC TTT ATT AAA CCT CTG AAA GCT AAC CAG CAA TTC TTA GTC TAC TOT						4736
Tyr Phe Ile Lys Pro Leu Lys Ala Asn Glu Glu Phe Leu Val Tyr Cys						
	195		200		205	
GAA ATC GAT GGG TCT GGA AAT GGA TGG ACT GTG TTT CAG AAG						4778
Glu Ile Asp Gly Ser Gly Asn Gly Trp Thr Val Phe Glu Lys						
	210		215		220	
GTAATTTTTT CCCCACCATG TGTATTTAAT AAATTCCTAC ATTGTTTCTG CCATATGACA						4838
GATACTTTTC TAAGCACCTT GTGAACCGTA GCTCATTTAA TCCTTGCAAT AGCCCTAAGA						4898
OGAAGGTACT TCTGTTACTC CTATTTACAG AAAAGGAAAC TGAAGGCACAC AAGGTTAAAT						4958
AACTTCCCCA AGACCACATA ACTAATAAGC AACAGAGTCA GCATTTGAAC CTAAGGACATA						5018
TAGTTTCAOA GTTTGTGACT TGACTCTATA TTGTACTGGC ACTGACTTTG TAGATTGATG						5078
GTGGCACATA ATCATAGTAC CACAOTGACA AATAAAAAAG AOGAAACTCT TTTGTCAOGT						5138
AGGTCAAGAC CTGAGGTTTC CCAICACAAG ATGAGGAAAGC CCAACACCAC CCCCCACCAC						5198
CCCACCACCA TCACCACCCT TTCACACACC AGAAGGATACA CTTGGGCTGC TCCAAGACAA						5258
GGAACCTGTG TTGCATCTGC CACTTGCTGA TACCCACTAG GAATCTTGGC TCCTTTACTT						5318
TCTGTTTACC TCCCACCACT GTTATAACTG TTTCTACAGG GGGCGCTCAG AOGGAAATGAA						5378
TGGTGGAAGC ATTAGTTGCC AGACACCGAT TGAOGCAATGG GTTCCATCAT AAGTGTAAQA						5438
ATCAGTAATA TCCAOGTAGA GTTCTGAAGT CGTCTAGGTG TCTTTTTAAT ATTACCACTC						5498
ATTTAGAAAT TATGATGTGC CAGAAACCCT CTTAAGTATT TCTCTTATAT TCTCTCTCAT						5558
GATCCTTGCA GCAACCCTAA GAAOTAACCA TCATTTTTCC TATTTGATAC ATGAGGAAAC						5618
TGAAGTAAGT TGGCCAAAGAT CACTTAGTTG GGAAGTTGATA GAACCAAGTGC TCTGTATTTT						5678
TGACAAAAATG TTGACAGCAT TCTCTTTACA TGCATTGATA GTCTATTTTC TCCTTTTGT						5738

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CTTGCAAATG TGTAAATTAG AGA CTT GAT GGC AGT GTA GAT TTC AAG AAA AAC	5790
Arg Leu Asp Gly Ser Val Asp Phe Lys Lys Asn	
225 230	
TGG ATT CAA TAT AAA GAA GGA TTT GGA CAT CTG TCT CCT ACT GGC ACA	5838
Trp Ile Glu Tyr Lys Glu Gly Phe Gly His Leu Ser Pro Thr Gly Thr	
235 240 245	
ACA GAA TTT TGG CTG GGA AAT GAG AAG ATT CAT TTG ATA AGC ACA CAG	5886
Thr Glu Phe Trp Leu Gly Asn Glu Lys Ile His Leu Ile Ser Thr Glu	
250 255 260 265	
TCT GCC ATC CCA TAT GCA TTA AGA GTG GAA CTG GAA GAC TGG AAT GGC	5934
Ser Ala Ile Pro Tyr Ala Leu Arg Val Glu Leu Glu Asp Trp Asn Gly	
270 275 280	
AGA ACC AG GTACTGTTTT GAAATGACTT CCAACTTTTT ATTGTAAAGA	5982
Arg Thr Ser	
TTGCCTGGAA TGTGCACCTT CCAACTATCA ATAGACAATG GCAAAATGCAG CCTGACAAAT	6042
GCAAAACAGCA CATCCAGCCA CCATTTTCTC CAGGAGTCTG TTTGGTTCTT GGGCAATCCA	6102
AAAAAGGTAAA TTCTATTGAG GATGAATCTA AGTGATTGAG TACAATCTAA TTACCTTGGG	6162
ACCATTGAGA GTAATAGCTA ATTACTGAAC TTTTAATCAG TCCCAGGAAT TGAACATAAA	6222
ATTATAATTT TATCTAGTCT AAATTACTAT TTCATGAAGC AGGTATTATT ATTAATCCCA	6282
TTTTATAGAT TAACTTGCTC AAAGTCACAT TGCTGATAAG TGGTAGAGGT AGAATTCAAG	6342
CTCAAAGTAGT TTAACCTTTAG AGCCTGTCTT CTAAACAACCT ATCCTGGTTG AAAAGCAAAAT	6402
ACAGCCTCTT CAGACTTCTC AGTGCCCTTGA TGGCCATTTA TTCTGTCAAA TCATGAGCTA	6462
CCCTAAAAGT AAACCAAGCTA GCTCTTTTGA TGATCTAGAG GCTTCTTTTT GCTTGAGATA	6522
TTTGAAGGTT TTAAGCATTG TTACCTAATT AAAATGCAGA AAAATATCCA ACCCTCTTGT	6582
TATGTTTAAAG GAATAGTGAA ATATATTGTC TTCAAACACA TGGACTTTTT TTTATTGCTT	6642
GGTTGGTTTT TAATCCAGAA AGTGCTATAG TCAGTAGACC TTCTTCTAGG AAAAGACCTT	6702
CCATTTCCCA GCCACTGAG ATTAGAAAAT AAGCTAAATA TTTTCTGGAA ATTTCTGTTC	6762
ATTCATTAAAG GCCCATCTT TCCCCACTC TATAGAAAGT TTGTCCACTT GCACAATTTT	6822
TTCCAGGAAA GAATCTCTCT AACTCCTTCA GCTCACATGC TTTGGACCAC ACAGGGAAAG	6882
CTTTGATTGT GTAATGCCCT CAGAAAGCTCT CCTTCTTGCC ACTACCACAC TGATTTGAGG	6942
AAOAAAATCC CTTTAGCACC TAACCCTTCA GGTGCTATGA GTGGCTAATG GAACTGTACC	7002
TCCTTCAAGT TTTGTGCAAT AATTAAGGAT CACTCACTGT CAGTACTTT CTGTGATCTA	7062
TGATAATGTG TGTGCAACAC ATAACATTTT AATAAAAAGTA GAAAATATGA AATTAGAGTC	7122
ATCTACACAT CTGGAATTTGA TCTTAAGAAAT AAACAAAGCA AAAAGCATCC AAGTGAAGTC	7182
AATTATTAGT TTTCAAGAGT GCTTCAAAAGG CTCTAGGACC CATCCCGGGA AGTGTTAATG	7242
AGCTGTGAGC TGGTTCACAT ATCTATTGCC TCTTGCCAGA TTTGCAAAAA ACTTCACTCA	7302
ATGAGCAAAAT TTCAGCCTTA AGAAACAAAAG TCAAAAATTTC CAAGGAAGCA TCCTACGAAA	7362
GAGGGAACTT CTGAGATCCC TGAAGAGGAT CAGCATGTGA TGGTTGTATT TCCTTCTTCT	7422
CAG T ACT GCA GAC TAT GCC ATG TTC AAG GTG GGA CCT GAA GCT GAC	7468
Thr Ala Asp Tyr Ala Met Phe Lys Val Gly Pro Glu Ala Asp	
285 290 295	
AAG TAC CCG CTA ACA TAT GCC TAC TTC GCT GGT GGG GAT GCT GGA GAT	7516
Lys Tyr Arg Leu Thr Tyr Ala Tyr Phe Ala Gly Gly Asp Ala Gly Asp	
300 305 310	
GCC TTT GAT GGC TTT GAT TTT GGC GAT GAT CCT AGT GAC AAG TTT TTC	7564
Ala Phe Asp Gly Phe Asp Phe Gly Asp Asp Pro Ser Asp Lys Phe Phe	
315 320 325 330	

65494.00 = 3342260

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ACA	TCC	CAT	AAT	GGC	ATG	CAO	TTC	AOT	ACC	TGG	GAC	AAT	GAC	AAT	GAT	7612
Thr	Ser	His	Asn	Gly	Met	Gln	Phe	Ser	Thr	Trp	Asp	Asn	Asp	Asn	Asp	
				335					340					345		
AAG	TTT	GAA	GGC	AAC	TGT	GCT	GAA	CAG	GAT	GGA	TCT	GGT	TGG	TGG	ATG	7660
Lys	Phe	Gln	Gly	Asn	Cys	Ala	Glu	Gln	Asp	Gly	Ser	Gly	Trp	Trp	Met	
			350					355					360			
AAC	AAG	TGT	CAC	GCT	GGC	CAT	CTC	AAT	GGA	GTT	TAT	TAC	CAA	G		7703
Asn	Lys	Cys	His	Ala	Gly	His	Leu	Asn	Gly	Val	Tyr	Tyr	Gln			
		365					370					375				
GTATGTTTTT	CTTTCTTAGA	TTCCAAGTIA	ATGTATAOTG	TATACTATTT	TCATAAAAAA											7763
TAATAAATAG	ATATGAAGAA	ATGAAGAATA	ATTTATAAAG	ATAGTAGGGA	TTTTATCATG											7823
TTCTTTATTT	CAACTAAGTT	CTTTGAAACT	GGAAOTGGAT	AATACCAAGT	TCATGCCTAA											7883
AATTAGCCCT	TCTAAAGAAA	TCCACCTGCT	GCAAAATATC	CAGTAGTTTG	GCATTATATG											7943
TGAAACTATC	ACCATCATAG	CTGGCACTGT	GGGTTGTGGG	ATCTCCTTTA	GACATACAAC											8003
ATAAATGATC	TGGATGGATT	AACATTACTA	CATGGATGCT	TGTTGACACA	TAAACCTGGC											8063
TTCCCATGAG	CTTTGTGTCA	GATACACGCA	GTGAACAAGT	GTTTGGAGGA	ACAGAATAAA											8123
GAGAAGGCCAA	GCACTGGTAA	GGGCAGGGGT	TGTGAAAAGC	TTGAGAGAAAG	AGACCAGTCT											8183
GAGGACAGTA	GACACTTATT	TTAGGATGGG	GGTTGGATGA	GGAGGCTATA	GTTTGTCTATA											8243
AGCTTGGAAAT	GGTTTGGAAC	ACTGGTTTCA	CTCACCTACC	CAGCAGTTAT	GTGTGGGGAA											8303
GCCTTACCGA	TGCTAAAAGGA	TCCATGTTAC	AATAATGGCA	TTATTTGGAA	ATCCCAOTGG											8363
TATTCCATGA	ATAAAACCAC	TATGAAAGATA	ATCCCACTCA	ACAGACTCTC	CGTTGGAGAA											8423
GGACAGCAAC	ACCACCCTGG	GAAAOCCTAA	CAGTCAGACC	AGACCTGTTT	AGCATCAOTA											8483
GGACTTCCCT	ACCATATCTG	CTGGGTAGAT	GAOTGAAAACC	AGTGTTCCAA	ACCACCTCCGG											8543
GCTTGTAGCA	AACCATAOTC	TCCTCATCTA	CCAAGATGAG	CAACCTTACC	TCCTGATGTC											8603
CTAGCCAATC	ACCAACTAGG	AAACTTTTGA	CAOTTTATTT	AAAGTAACAG	TTTGTATTTT											8663
ACAATATTTT	TAAATTGGAG	AAACATAACT	TATCTTTTGA	CTCACAAACC	ACATAATGAG											8723
AAAGAACTCT	AAGGGAAAAAT	GCTTGTATCTG	TGTGACCCGG	GGCGCCATGC	CAGAGCTGTA											8783
GTTTCATGCCA	GTGTTGTGCT	CTGACAAOCC	TTTTACAGAA	TTACATGAGA	TCTGCTTCCC											8843
TAGGACAAAG	AGAAOGCAAA	TCAACAAGAG	CTOCACTTTA	AAATGGAGAC	ATAAAATAAC											8903
ATGCCAGAAC	CATTTCTTAA	AGCTCCTCAA	TCAACCAACA	AAATTGTGCT	TTCAAAATAAC											8963
CTOAGTTGAC	CTCATCAOGA	ATTTTGTGGC	TCCTTCTCTT	CTAACCTGCC	TGAAGAAAGA											9023
TGGTCCACA	CAGCTGAGTC	COGGATGGAT	AAOCTTAGGG	ACAGAOGCCA	ATTAGOGAAC											9083
TTTGGGTTTT	TAGCCCTACT	AGTAGTGAAT	AAATTTAAAA	TGTGGATGTG	ACTATGAGTC											9143
ACAGCACAGA	TGTTGTTTTA	TAATATGTTT	ATTTTATAAA	TTGATATTTT	AGGAATCTTT											9203
GAAGATATTT	TCAOTTAGCA	GATAATACTA	TAAATTTTAT	GTAACCTGGCA	ATGCACCTCG											9263
TAATAGACAG	CTCTTCATAG	ACTTGCAGAG	GTAAAAAGAT	TCCAGAAATA	TGATATGTAC											9323
ATCTACGACT	TGTTTTAG	GT GGC ACT	TAC TCA AAA	GCA TCT ACT	CCT AAT											9373
		Gly Gly Thr	Tyr Ser Lys Ala Ser	Thr Pro Asn												
			380		385											
GGT TAT GAT	AAT GGC ATT	ATT TGG	OCC ACT TGG	AAA ACC CGG	TGG TAT											9421
Gly Tyr Asp	Asn Gly Ile	Ile Trp	Ala Thr Trp	Lys Thr Arg	Trp Tyr											
	390		395		400											
TCC ATG AAG	AAA ACC ACT	ATG AAG	ATA ATC CCA	TTC AAC AGA	CTC ACA											9469
Ser Met Lys	Lys Thr Thr	Met Lys	Ile Ile Pro	Phe Asn Arg	Leu Thr											
	405		410		415											
ATT GGA GAA	GGA CAG CAA	CAC CAC	CTG GGG GGA	GCC AAA CAG	GTC AGA											9517

GCTT = 244220

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Ile	Gly	Glu	Gly	Gln	Gln	His	His	Leu	Gly	Gly	Ala	Lys	Gln	Val	Arg	
420					425					430					435	
CCA	GAG	CAC	CCT	GCG	GAA	ACA	GAA	TAT	GAC	TCA	CTT	TAC	CCT	GAG	GAT	9565
Pro	Glu	His	Pro	Ala	Glu	Thr	Glu	Tyr	Asp	Ser	Leu	Tyr	Pro	Glu	Asp	
			440					445					450			
GAT	TTG	TAGAAAATTA	ACTGCTAACT	TCTATTGACC	CACAAAAGTTT	CAGAAATTCT										9621
Asp	Leu															
CTGAAAAGTTT	CTTCCTTTTT	TCTCTTACTA	TATTTATTGA	TTTCAAGTCT	TCTATTAAGG											9681
ACATTTAGCC	TTCAATGGAA	ATTAAAACTC	ATTTAGGACT	GTATTTCCAA	ATTACTGATA											9741
TCAGAGTTAT	TTAAAAATTG	TTTATTTGAG	GAGATAACAT	TTCAACTTTG	TTCCTAAATA											9801
TATAATAATA	AAATGATTGA	CTTTATTTTC	ATTTTTATGA	CCACTTGTCA	TTTATTTTGT											9861
CTTCGTAAAT	TATTTTCATT	ATATCAAATA	TTTTAGTATG	TACTTAATAA	AATAGGAGAA											9921
CATTTTAGAG	TTTCAAATTC	CCAAGTATTT	TCCTTGTTTA	TTACCCCTAA	ATCATTCTTA											9981
TTTAAATCTT	CTTTTTAAAT	GGAGAAAAAT	ATGTCTTTTT	AATATGGTTT	TTGTTTTGTT											10041
ATATATTCAC	AGGCTGGAGA	CGTTTAAAAAG	ACCGTTTCAA	AAGAGATTTA	CTTTTTTAAA											10101
GGACTTTATC	TGAACAGAGA	GATATAATAT	TTTTCTTATT	GGACAATGGA	CTTGCAAAGC											10161
TTCACTTCAT	TITAAGAGCA	AAAGACCCCA	TGTTGAAAAC	TCCATAACAG	TTTTATGCTG											10221
ATGATAATTT	ATCTACATGC	ATTTCAATAA	ACCTTTTGTT	TCCTAAGACT	AGATACATGG											10281
TACCTTTATT	GACCATTAIAA	AAACCACCAC	TTTTTGCCAA	TTTACCAATT	ACAATTGGGC											10341
AACCATCAAT	AGTAATTGAG	TCCTCATTTT	ATGCTAAATG	TTATGCCTAA	CTCTTTGGGA											10401
GTTACAAAAG	AAATAGCAAT	TATGGCTTTT	GCCCTCTAGG	AGATACAGGA	CAAATACAGG											10461
AAAATACAGC	AACCCAAACT	GACAATACTC	TATACAAGAA	CATAATCACT	AAGCAGGAGT											10521
CACAAGCCACA	CAACCAAGAT	GCATAGTATC	CAAAGTGACG	CTG												10564

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 453 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Ser	Trp	Ser	Leu	His	Pro	Arg	Asn	Leu	Ile	Leu	Tyr	Phe	Tyr	Ala	
1				5					10					15		
Leu	Leu	Phe	Leu	Ser	Ser	Thr	Cys	Val	Ala	Tyr	Val	Ala	Thr	Arg	Asp	
		20						25					30			
Asn	Cys	Cys	Ile	Leu	Asp	Glu	Arg	Phe	Gly	Ser	Tyr	Cys	Pro	Thr	Thr	
		35					40					45				
Cys	Gly	Ile	Ala	Asp	Phe	Leu	Ser	Thr	Tyr	Gln	Thr	Lys	Val	Asp	Lys	
	50					55					60					
Asp	Leu	Gln	Ser	Leu	Glu	Asp	Ile	Leu	His	Gln	Val	Glu	Asn	Lys	Thr	
	65				70					75				80		
Ser	Glu	Val	Lys	Gln	Leu	Ile	Lys	Ala	Ile	Gln	Leu	Thr	Tyr	Asn	Pro	
			85					90						95		
Asp	Glu	Ser	Ser	Lys	Pro	Asn	Met	Ile	Asp	Ala	Ala	Thr	Leu	Lys	Ser	
		100					105						110			
Arg	Ile	Met	Leu	Gln	Glu	Ile	Met	Lys	Tyr	Gln	Ala	Ser	Ile	Leu	Thr	
	115					120						125				
His	Asp	Ser	Ser	Ile	Arg	Tyr	Leu	Gln	Glu	Ile	Tyr	Asn	Ser	Asn	Asn	
	130					135					140					

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Gln	Lys	Ile	Val	Asn	Leu	Lys	Glu	Lys	Val	Ala	Gln	Leu	Glu	Ala	Gln
145					150					155					160
Cys	Gln	Gln	Pro	Cys	Lys	Asp	Thr	Val	Gln	Ile	His	Asp	Ile	Thr	Gly
				165					170					175	
Lys	Asp	Cys	Gln	Asp	Ile	Ala	Asn	Lys	Gly	Ala	Lys	Gln	Ser	Gly	Leu
			180					185					190		
Tyr	Phe	Ile	Lys	Pro	Leu	Lys	Ala	Asn	Gln	Gln	Phe	Leu	Val	Tyr	Cys
		195					200					205			
Glu	Ile	Asp	Gly	Ser	Gly	Asn	Gly	Trp	Thr	Val	Phe	Gln	Lys	Arg	Leu
	210					215					220				
Asp	Gly	Ser	Val	Asp	Phe	Lys	Lys	Asn	Trp	Ile	Gln	Tyr	Lys	Gln	Gly
225					230					235					240
Phe	Gly	His	Leu	Ser	Pro	Thr	Gly	Thr	Thr	Gln	Phe	Trp	Leu	Gly	Asn
				245					250					255	
Glu	Lys	Ile	His	Leu	Ile	Ser	Thr	Gln	Ser	Ala	Ile	Pro	Tyr	Ala	Leu
			260					265					270		
Arg	Val	Gln	Leu	Gln	Asp	Trp	Asn	Gly	Arg	Thr	Ser	Thr	Ala	Asp	Tyr
		275					280					285			
Ala	Met	Phe	Lys	Val	Gly	Pro	Gln	Ala	Asp	Lys	Tyr	Arg	Leu	Thr	Tyr
	290					295					300				
Ala	Tyr	Phe	Ala	Gly	Gly	Asp	Ala	Gly	Asp	Ala	Phe	Asp	Gly	Phe	Asp
305					310					315					320
Phe	Gly	Asp	Asp	Pro	Ser	Asp	Lys	Phe	Phe	Thr	Ser	His	Asn	Gly	Met
				325					330					335	
Gln	Phe	Ser	Thr	Trp	Asp	Asn	Asp	Asn	Asp	Lys	Phe	Gln	Gly	Asn	Cys
			340					345					350		
Ala	Gln	Gln	Asp	Gly	Ser	Gly	Trp	Trp	Met	Asn	Lys	Cys	His	Ala	Gly
		355					360					365			
His	Leu	Asn	Gly	Val	Tyr	Tyr	Gln	Gly	Gly	Thr	Tyr	Ser	Lys	Ala	Ser
	370					375					380				
Thr	Pro	Asn	Gly	Tyr	Asp	Asn	Gly	Ile	Ile	Trp	Ala	Thr	Trp	Lys	Thr
385					390					395					400
Arg	Trp	Tyr	Ser	Met	Lys	Lys	Thr	Thr	Met	Lys	Ile	Ile	Pro	Phe	Asn
				405					410					415	
Arg	Leu	Thr	Ile	Gly	Gln	Gly	Gln	Gln	His	His	Leu	Gly	Gly	Ala	Lys
			420					425					430		
Gln	Val	Arg	Pro	Gln	His	Pro	Ala	Gln	Thr	Gln	Tyr	Asp	Ser	Leu	Tyr
		435					440					445			
Pro	Gln	Asp	Asp	Leu											
				450											

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10807 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ovine beta- lactoglobulin

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACGCGTGTCTG	ACCTGCAGGT	CAACGGATCT	CTGTGTCTGT	TTTCATGTTA	GTACCACACT	60
GTGTTGGTGG	CTGTAGCTTT	CAGCTACAGT	CTGAAGTCAT	AAAAGCCTGGT	ACCTCCAAGT	120
CTGTTCTCTC	TCAAGATTGT	GTTCTGCTGT	TTGGGTCTTT	AGTGTCTCCA	CACAATTTT	180

AGAAATTGTTT	GTTCTAGTTT	TGTGAAAAAT	GATGCTGGTA	TTTTGATAAG	GATTGCATTG	240
AATCTGTAAA	GCTACAGATA	TAGTCATTGG	GTAGTACAGT	CACTTTAACA	ATATTAACTC	300
TTACACATCTG	TGAGCATGAT	ATATTTTCCC	CCTCTATATC	ATCTTCAATT	CCTCCTATCA	360
GTTTCTTTCA	TTGCAGTTTT	CTGAGTACAG	GTCTTACACC	TCCTTGGTTA	GAGTCATTCC	420
TCAGTATTTT	ATTCCTTTGA	TACAATTGTG	AATGAGGTAA	TTTTCTTAGT	TTCCTTTTCT	480
GATAGCTCAT	TGTTAGTGTA	TATATAGAAA	AGCAACAGAT	TTCTATGTAT	TAATTTTGTA	540
TCCTGCAACA	GATTTCTATG	TATTAATTTT	GTATCCTGCT	ACTTTACGGA	ATTCACTTAT	600
TAGCTTTTTG	GTGACATCTT	GAGGATTTTC	TGAAGAAAAAT	GGCATGGTAT	GGTAGGACAA	660
GGTGTCATGT	CATCTGCAAA	CAGTGGCAGT	TTTCCTTCTT	CCCTTCCAAC	CTGGATTTCT	720
TTGATTTCTT	TCTGTCTGAG	TACGACTAGG	ATTCCCAATA	CTATACCGAA	TAAAAGTGGC	780
AAGAGTGGAC	ATCCTTTGCT	TATTTTTCTG	ACCTTAGAGG	AAATGCTTTC	AGTTTTTCAC	840
CATTAATTAT	AATGTTTACT	GTGGGCTTGT	CATATGTGGC	CTTCATTATA	TGGAGGTCTA	900
TTCCCTCTAT	ACCCACCTTG	TTGAGAGTTT	TTATCATAAA	AGTATGTTGA	ATTTTGTCAA	960
AAGTTTTTTC	TGCATCTATT	GAGATGATTT	TTACTCTTCA	ATTCATTAAT	GATTTTTTATT	1020
CTTCATTTTG	TTAATGATTT	CCATTCTTCA	ATTTGTTAAC	GTGGTATATC	ACATTGATTG	1080
ATTTGTGGAT	ACCTTTGTAT	CCCTGGGATA	AACCTCACAT	GATCATGAGC	TTTCAATGTA	1140
TTTTTTGAATT	CACTTTGCTA	ATATTCTGTT	GGGTATTTTT	GCATCTCTAT	TCATCAATGA	1200
TATTGOCCTA	AGAAAAGTTT	TGTCTGGTTT	TAGTATCAGG	GTGATGCTGG	CCTCATAGAG	1260
AGAGTTTAGA	AGCATTTCCT	CCTCTTTGAT	TTTTCGGAAT	AGTTTGAGTA	GGATAAGGTAT	1320
TAACCTCTCT	TTAAATGTTT	GGGGACTTCC	CTGGTGAGCC	GGTGOTTGAG	AATCCGCCCTC	1380
AGGGATGTGG	GTTTGATCCC	TGGTCAAGGA	ACCATTAATA	AGATCCCACA	TGCTGCAGGC	1440
AACAAOCCCC	CAAAGCTGCA	CCACTGAGCT	GCAACCCTG	CAGTGCCAC	AGGCCACGAC	1500
CAGAGAAAAG	CCACATACAG	CAGGGAAGAC	CCAGCACAAAC	CAGAAAAAG	AGTTTGGTGG	1560
AATACAGCTG	TGAAGCCGTC	TGGTCCTGGA	CTCCTGCTTG	AGGGAATTTT	TTAAAAATTA	1620
TTGATTCAAT	TTCAATTACTG	GTAACGTGTC	TGTTCATATT	TTCTATTTCT	TCCGGGTTCA	1680
GTCTTGGGAG	ATTGTACATG	CCTAAGGAATG	TGTCCGTTTC	TTCTAAGTTG	TCCATTTTAT	1740
TGGACATGCA	TGGGAACACA	CAGCACCGAC	CAGCGAGACT	CATGCTGGCT	TCCTGGGGCC	1800
AGGCTGGGGC	CCCAAACAGC	ATGGCATCCT	AGAATGTGTG	AAAGCCCACT	GACCCCTGCCC	1860
AGCCCCACAA	TTTCATTCTG	AGAAATGATT	CTTGTCTTCT	GCATTACAG	GCCCCAGGATC	1920
TGACCTGCTT	CTAAGGAGCA	GGGGTTTTGG	CAGGACGGGG	AGATGCTGAG	AGCCGACGGG	1980
GGTCCAAGTC	CCCTCCCAAG	CCCCCTGTG	TGGGGCAGCC	CTTGGGAAAG	ATTGCCCCAG	2040
TCTCCCTCCT	ACAATGTTCA	GTCCCAAGCTG	CCCCAGGCCA	GAGCTGCTTT	ATTTCCGTCT	2100
CTCTCTCTGG	ATGGTATTCT	CTGGAAGCTG	AAAGTTTCTG	AAGTTATGAA	TAAGTTTGGC	2160
CTGAAGGGCA	TGGTTTGTGG	TCACGGTTCA	CAGGAACITG	GGAGACCCTG	CAGCTCAGAC	2220
GTCCCGAGAT	TGGTGGCACC	CAGATTTCCT	AAGCTCGCTG	GGGAACAAGG	CGCTTGTTC	2280
TCCCTGGCTG	ACCTCCCTCC	TCCCTGCATC	ACCCAGTTCT	GAAAGCAGAG	CGGTGCTGGG	2340
GTCAACAACCT	CTCGCATCTA	ACGCCGGGTG	CCAAACCACC	CGTGCTGGTG	TTGGGGGGGC	2400
TACCTATGGG	GAAAGGGCTTC	TCACTGCAAT	GGTGCCCCCC	GTCCCCCTCTG	AGATCAAGAG	2460
TCCCACTCCG	GACGTCAAAC	AGGCCGAGCT	CCCTCCAGAG	GCTCCAGGGA	GGGATCCTTG	2520
CCCCCCCCGT	GCTGCTTCCA	GCTCCTGGTG	CCGCACCCCT	GAOCTGATC	TTGTAGACGC	2580

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CTCAGTCTAG	TCTCTGCCTC	COTGTTTACA	COCCTTCTCC	CCATGTCCCC	TCCGTGTCCC	2640
CGTTTTCTCT	CACAAAGGACA	CCGGACATTA	GATTAGCCCC	TGTTCCAGCC	TCACCTGAAC	2700
AGCTCACATC	TGTAAAGACC	TAGATTCCAA	ACAAAGATTCC	AACCTGAAGT	TCCCGGTGGA	2760
TGTGAGTTCT	GGGGCGACAT	CCTTCAACCC	CATCACAGCT	TGCAGTTTAT	CGCAAAACAT	2820
GGAAACCTGG	GTTTATCGTA	AAACCCAGGT	TCTTCATGAA	ACACTGAGCT	TGAGGGCTTG	2880
TTGCAAGAAAT	TAAAGGTGCT	AATACAGATC	AAGGCAAGGA	CTGAAAGCTG	CTAAGCCTCC	2940
TCITTTCCATC	ACAGGAAAAG	GGGGCCTGGG	GGCGGCTGGA	GGTCTGCTCC	CGTGAAGTGA	3000
CTCTTTCCCTG	CTACAGTCAC	CAACAGTCTC	TCTGGGAAAG	AAACCAGAGG	CCAGAGAGCA	3060
AGCCGGAGCT	AGTTTAGGAG	ACCCCTGAAC	CTCCACCCAA	GATGCTGACC	AGCCAAGCGGG	3120
CCCCCTGGAA	AGACCCTACA	GTTCAGGGGG	GAAAGAGGGG	TGACCCGCCA	GGTCCCTGCT	3180
ATCAGGAGAC	ATCCCCGCTA	TCAAGAGATT	CCCCCACCTT	GCTCCCGTTC	CCCTATCCCA	3240
ATACGCCCCAC	CCCACCCCTG	TGATGAAGCA	TTTAGTCACT	TAGAAATGTCA	ACTGAAGGCT	3300
TTTGCAATCC	CTTTGCCAGA	GGCACAAGGC	ACCCACAGCC	TGCTGGGTAC	CGACGCCCCAT	3360
GTGGATTCA	CCAGGAGGGC	TGTCTCTCAC	CCTCCCTGCT	CGGGCCCCCT	CTGTGCTCA	3420
CAACACACCC	AGCACCAAGCA	TTCCCGCTGC	TCCTGAGGTC	TGCAAGCAGC	TGCTGTATGC	3480
CTGAGCGGTG	TGGAAGGAA	TGTCTCTGGA	GATTIAAAAT	GTGAAGAGCG	GGAGGTGGGA	3540
GGTTGGGGCC	TGTGGGCCCTG	CCCATCCCAC	GTGCTGTCAT	TAGCCCCAGT	GCTGCTCAAG	3600
CGTGCCCCCG	CCGCAAGGGT	CAAGTCACTT	TCCCGTCTCT	GGGTTATTAT	GACTCTTTGT	3660
ATTGCCATTG	CCATTTTTGC	TACCCTAACT	GGGCAAGCAG	TGCTTGCAAG	GGCCTCGATA	3720
CCGACCAGGT	CCTCCCTCGG	AGCTCGACCT	GAACCCCATG	TCACCCCTGC	CCCAGCCTGC	3780
AGAAGGGTGG	TGACTGCAAG	GATCCCTTCA	CCCAAGGCCA	CGGTTCACAT	GTTTGGAGGA	3840
GCTGGTGCCC	AAGGCAGAGG	CCACCCCTCA	GGACACACCT	GTCCCGAGTG	CTGGCTCTGA	3900
CCTGTCTTGG	TCTAAGAGGC	TGACCCCGGA	AGTGTTCCTG	GCACTGGCAG	CCAAGCCTGA	3960
CCCAGAGTCC	AGACACCCAC	CTGTGCCCCC	GCTTCTGGGG	TCTACCAAGG	ACCCTCTAGG	4020
CCCAGAGGGG	ACTTCTGCTG	TGGCCTTGGG	TGGAAGAAAG	CCTCCTATTG	TCCTCGTAGA	4080
GGAAAGCCACC	CCGGGGCCTG	AGGATGAGCC	AAGTGGGATT	CCGGGAACCG	CGTGGCTGGG	4140
GGCCCCAGCC	GGGCTGGCTG	GGCTGTCATG	CTCCTGTATA	AAGCCCCAAG	CCTGCTGTCT	4200
CAGCCCTCCA	CTCCCTGCA	AGCTCAGAGG	CACGACCCCA	GGGATATCCC	TGCAAGCCATG	4260
AAGTGCCCTC	TGCTTGCCCT	GGGCTTGGCC	CTGCGCTGTG	GGGTCCAGGC	CATCATCGTC	4320
ACCCAGACCA	TGAAAAGGCCT	GGACATCCA	AAAGTTTCAG	GGTTGGCCGG	GTGGGTGAAT	4380
TGCAAGGGCG	GCAGGGGAGC	TGGGCCCTCA	AGAAGCCAAAG	GAAGCTGTGA	CGTTGGGTTC	4440
CCATCAGTCA	GCTAGGGCCA	CCTGACAAAT	CCCCGCTGGG	GCAAGCTTCA	CCAGGCGTTC	4500
ACTGTCTTGG	ATTCTGGAAG	CTGGAAGGCC	AAGATCCAGG	TGTTGGCAAG	GCTGGCTTCT	4560
CCTGCGGGCG	CTCTCTGGGG	AOCAGACGGC	CGTCTTCTCC	AGTCTCTGCT	GGCGCCCTGAT	4620
TTCTCTTTC	TGTGAGGCCA	CCAAGCCTGC	TGGAAGACAG	CCTGGCTGCG	CAAGCTTACA	4680
CGACCTTTGT	CATCTCTTTA	AAGGCCATGT	CTCCAGAGTC	ATGTGTTGAA	GTCTGGGGGG	4740
TTAGTGGGAC	ACAGTTCAAG	CCCTAAAAGA	GTCTCTCTGC	CCCTCAAAAT	TTCCCCACCT	4800
CCAGCCATGT	CTCCCCAAGA	TCCAAATGTT	GCTACATGTG	GGGGGGCTCA	TCTGGGTCCC	4860
TCTTTGGGTT	CAGTGTGAAT	CTGGGAGAGG	CATTCGCCAG	GGTGCAAGAT	TGGGGGGAGT	4920
ATCTCAGGGC	TGCCCAGGCC	GGGGTGGGAC	AGAAGAGCCCA	CTGTGGGGCT	GGGGGGCCCC	4980

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TCCCCACCC	AGAATGCAAC	TCAAAGTCCC	TCTCCAGGTG	GCAGGAGACTT	GGCACTCCTT	5040
GGCTATGGC	GCCAGCGACA	TCTCCCTGCT	GGATGCCAG	AGTGCCCCC	TGAGAGTGTA	5100
CGTGGAGGAG	CTGAAAGCCCA	CCCCCGAGGG	CAACCTGGAG	ATCCTGCTGC	AGAAATGGTG	5160
GGCGTCTCTC	CCCAACATGG	AACCCCCACT	CCCCAGGGCT	GTGGACCCCC	CGGGGGGTGG	5220
GGTGCAAGGAG	GGACCAAGGGC	CCCAGGGCTG	GGGAAAGAGG	CTCAGAGTTT	ACTGGTACCC	5280
GGCGCTCCAC	CCAAAGGCTGC	CCACCCAGGG	CTTTTTTTTT	TTTTAAACTT	TTATTAATTT	5340
GATGCTTCAG	AACATCATCA	AACAAATGAA	CATAAAACAT	TCATTTTTGT	TTACTTGAAA	5400
GGGAGATAA	AATCCTCTGA	AGTGGAAATG	CATAGCAAA	ATACATACAA	TGAGGCAAGT	5460
ATTCTGAATT	CCCTGTTAGT	CTGAGGATTA	CAAGTGTATT	TGAGCAACAG	AGAGACATTT	5520
TCATCATTTT	TAGTCTGAAC	ACCTCAGTAT	CTAAAATGAA	CAAGAAAGTCC	TGGAAACGAA	5580
GCAGTGTGGG	GATAAGCCCCG	TGTGAAGGCT	GCTGGGAGGC	AGCAGACCTG	GGTCTTCGGG	5640
CTCAAAGCAGT	TCCCAGCTACC	AGCCCTGTCC	ACCTCAGACG	GGGGTCAGGG	TGCAGGAGAG	5700
AGCTGGATGG	GTGTGGGGGC	AGAGATGGGG	ACCTGAACCC	CAGGGCTGCC	TTTTGGGGGT	5760
GCCTGTGGTC	AAGGCTCTCC	CTGACCTTTT	CTCTCTGGCT	TCATCTGACT	TCTCCTGGCC	5820
CATCCACCCG	GTCCCCTGTG	GCCTGAGGTG	ACAGTGAAGT	CGCCGAGGCT	AGTTGGCCAG	5880
CTGGCTCCTA	TGCCCATGCC	ACCCCCCTCC	AGCCCTCCTG	GGCCAGCTTC	TGCCCTGGC	5940
CCTCAATTCA	TCCTGATGAA	AATGGTCCAT	GCCAATGGCT	CAGAAAGCAG	CTGTCTTTCA	6000
GGGAGAACGG	CGAGTGTGCT	CAAGAAAGAA	TTATTGCAGA	AAAAACCAAG	ATCCCTGCGG	6060
TGTTCAAGAT	CGATGGTGAG	TCCGGGTCCC	TGGGGGACAC	CCACCACCCC	CGCCCCGGGG	6120
GACTGTGGAC	AGGTTCAAGG	GGCTGGCGTC	GGGCCCTGGG	ATGCTAAGGG	ACTGGTGGTG	6180
ATGAAGACAC	TGCCCTTGACA	CCTGCTTCAC	TTGCCCTCCC	TGCCACCTGC	CCGGGGCCTT	6240
GGGGCGGTGG	CCATGGGACG	GTCCCAGGCT	GGGGCTAAC	CCACCAGGGT	GACACCCGAG	6300
CTCTCTTTGC	TGGGGGGCGG	GGGGTGGCTT	GGGCCCTCAG	GCTGAGCTCA	GGAGGTACCT	6360
GTGCCCTCCC	AAGGGTAACC	GAGAGCCGTT	GGCCACTCCA	GGGGCCAGGG	TGCCCCACGA	6420
CCCCAGCCCC	CTCCACAGCT	CCTTCATCTC	CTGAGAGCAA	ACTCTGTCCG	CCCTCGCTCA	6480
TTCACTTGT	CGTCTAAAT	CCGAGATGAT	AAAAGCTTCGA	GGGGGGGTGG	GGGTTCATC	6540
AGGGCTGCCC	TTCCGGCGGG	CAGCCTGGGC	CACATCTGCC	CTTGGCCCCC	TCAAGGACTCA	6600
CTCTGACTGG	AGGCCCTGCA	CTGACTGACG	CCAGGGTGGC	CAGCCAGGGG	TCTCTGGCGC	6660
CATCCAGCTG	CACTGGGTTT	GGGTGCTGGT	CCTGCCCCCA	AGCTGCCCCG	ACACCACAGG	6720
CAGCCGGGGC	TGCCCACTGG	CCTCGGTGAG	GGTGAAGCCCC	AGCTGCCCCC	GCTCAAGGGCT	6780
TGCCCCGACA	ATGACCCCAT	CCTCAAGAGC	CACCCCCCTT	CCCTTGGCTGG	GCAAGTGTCCA	6840
GGCCCCACCC	AGATCGGGGG	AAGCCCTATT	TCTTGACAAC	TCCAATCCCT	GGGGGAGGGG	6900
GCCTCAGACT	GAGTGGTGAG	TGTTCCCAA	TCCAGGAGGT	GGTGAAGGGT	CCTGGCGGAT	6960
CCAGAGTTGA	CAGTGAAGGC	TTCTGGGGCC	CCATGCGGCT	GGCAGTGGCA	GCAAGGAGAA	7020
GGAAAGCACC	TTTCAAGGGT	GGGGGATGCC	AGAAGCGCTC	CCCACCCCGT	CTTCGCCGGG	7080
TGGTGACCCC	GGGGGAGGCC	CGCTGGTCTG	GGAGGGTGGT	GGGGGCTGAC	TAGCAACCCC	7140
TCCCCCCCCG	TTGGAAGTCA	CTTTTCTCCC	GTCTTGACCG	CGTCCAAGCT	TGAATGAGAA	7200
CAAAATCCTT	GTGCTGGACA	CCGACTACAA	AAAATACCTG	CTCTTCTGCA	TGAAAAACAG	7260
TGCTGAAGCC	GAGCAAAAGCC	TGGCCTGCCA	GTGCTGGGGT	GGGTGCCAAC	CCTGGCTGCC	7320
CAGGGAGACC	AGCTGCGTGG	TCCTTGGCTG	AACAGGGGGT	GGGGGGTGGG	AGCTTGATCC	7380

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CCAGGAGGAG	GAAGGGGTGG	GGTCCCTGA	GTCCCGCCAG	GAGAGAGTGG	TCGCATACCG	7440
GGAGCCAGTC	TGCTGTGGGC	CTGTGGGTGG	CTGGGGACGG	GGGCCAGACA	CACAGGCCGG	7500
GAGACGGGTG	GGCTGCAGAA	CTGTGACTGG	TGTGACCGTC	GCATGGGGGC	CGGTGGTCAC	7560
TGAATCTAAC	AGCCTTTGTT	ACCGGGGAGT	TTCAATTATT	TCCCAAAATA	AGAACTCAAG	7620
TACAAAGCCA	TCTTTCAACT	ATCACATCCT	GAAAACAAAT	GGCAGGTGAC	ATTTTCTGTG	7680
CCGTAGCAGT	CCCAGTGGGC	ATTTTCAAGG	CCCCTGTGCC	AGGGGGGGCG	GGGCATCGGC	7740
GAGTGGAGGC	TCCTGGCTGT	GTGAGCCGGC	CCAGGGGGAG	GAAGGGACCC	GGACAGCCAG	7800
AGGTGGGGGG	CAGGCTTTCC	CCCTGTGACC	TGCAGACCCA	CTGCACTGCC	CTGGGAGGAA	7860
GGGAGGGGAA	CTAGGCCAAAG	GGGGAGGGGC	AGGTGCTCTG	GAGGGCAAGG	GCAGACCTGC	7920
AGACCACCCT	GGGGAGCAGG	GACTGACCCC	CGTCCCTGCC	CCATAGTCAG	GACCCCGGAG	7980
GTGGACAACG	AGGCCCTGGA	GAAATTGAGC	AAAAGCCCTCA	AGGCCCTGCC	CATGCACATC	8040
CGGCTTGCCCT	TCAACCCGAC	CCAAGCTGAG	GGTGAAGCACC	CAGGCCCGGC	CCTTCCCCAG	8100
GGCAAGAGCC	ACCCGGCCCC	GGGACGACCT	CCTCCCATGG	TGACCCCAAG	CTCCCAAGGC	8160
CTCCCAAGAG	GAAGGGGTGG	GGTGCAGCAC	CCCCTGGGGG	CCCCCTCCCC	ACCCCTTCCC	8220
AGGCCTCTCT	TCCCGAAGTG	TCCAGTCCCA	TCCTGACCCC	CCCATGACTC	TCCCTCCCCC	8280
ACAAGGCAAGT	GCCACGTCTA	GGTGAAGCCCC	TGCCGGTGCC	TCTGGGGTAA	GCTGCCTGCC	8340
CTGCCCCACG	TCCTGGGCAC	ACACATGGGG	TAAGGGGTCT	TGGTGGGGCC	TGGGACCCCA	8400
CATCAGGCCC	TGGGGTCCCC	CCTGTGAAGAA	TGGCTGGAAG	CTGGGGTCCC	TCCTGGGCGAC	8460
TGCAGAGCTG	GCTGGCCCGG	TGCCACTCTT	GTGGGTGACC	TGTGTCTCTG	CCTCACACAC	8520
TGACCTCCTC	CAGCTCCTTC	CAGCAGAGCT	AAAGCTAAAT	GAGCCAGAAAT	GGTACCTAAG	8580
GGGAGGCTAG	CGGTCTTTCT	CCCAGGGAAG	GGCTGTCTCT	GAACCAACAG	CCATGGAGAG	8640
GCTGGCAAGG	GTCTGGCAAG	TGCCCCAGGA	ATCACAGGGG	GGCCCCATGT	CCATTTCAAG	8700
GCCCCGGAGC	CTTGGACTCC	TCTGGGGACA	GACGACGTCA	CCACCGCCCC	CCCCCATCA	8760
GGGGGACTAG	AAAGGAGCAG	GACTGCAATC	ACCTTTCCTG	GGACCAAGGC	CCCTCCAAGC	8820
CCCTCCTGGG	GCTCCTGCTC	TGGGCAAGCTT	CTCCTTCACC	AATAAAGGCA	TAAACCTGTG	8880
CTCTCCCTTC	TGAGTCTTTG	CTGGAGCAGC	GGCAAGGGGT	GGAGAAAGTG	TGGGGAGGGA	8940
GTCTGGCTCA	GAGGATGACA	GGGGGGCTGG	GATCCAGGGC	GTCTGCATCA	CAGTCTTGTG	9000
ACAAGTGGGG	GCCCACACAC	ATCACTGCGG	CTCTTTGAAA	CTTTCAGGAA	CCAGGGAGGG	9060
ACTCGGCAAG	GACATCTGCC	AGTTCACTTG	GAAGTTTCAAG	TCAACACCCA	AACTCGACAA	9120
AGGACAGAAA	GTGGAAAAATG	GCTGTCTCTT	AGTCTAATAA	ATATTGATAT	GAAACTCAAG	9180
TTGCTCATGG	ATCAATATGC	CTTTATGATC	CAAGCCAGCCA	CTACTGTCTG	ATCAACTCAT	9240
GTACCCAAAC	GCAGTGAATC	GTCTGGCTAA	TGATGAGAGG	TTCCCAAGTAG	AGAGCTGGCA	9300
AGAAGTCACA	GTGAGAACTG	TCTGCACACA	CAAGCAGATC	CACCAATCAT	CCTAAGGAGG	9360
TCAGTCTCTG	TGTTCAATGG	AGGACTGATG	TTGAAAGCTGA	AACTCCAATG	CTTTGGCCAC	9420
CTGATGTGAA	GAGCTGACTC	ATTTGAAAAAG	ACCCTGATGC	TGGGAAAGAT	TGAGGGCAGG	9480
AGGAGAAAGG	GACGACAGAG	GATGAGATGG	TTGGATGGCA	TCACCAACAC	AATGGACATG	9540
GGTTTGGGTG	GACTCCAGGA	GTGGGTGATG	GACAAGGAGG	CCTGGCGTGC	TACGGAAAGC	9600
GTTTATGGGG	TCACAAAGAC	TGAGTGACTG	AACTGAGCTG	AACTGAATGG	AAATGAGGTA	9660
TACAGCAAGG	TGGGGATTTT	TTAGATAATA	AGAAATATACA	CATAACATAG	TGTATACTCA	9720
TATTTTATGG	CATACCTGAA	TGCTCAATCA	CTCAGTCGTA	TCTGACTCTG	TGACCTATGG	9780

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ACCGTAGCCT	TCCAGGTTTC	TTCTGTCCAC	AGAATTCTCC	AAGGCAAGAA	TACTGGAGTG	9840
GGTAGCCATT	TCCTCCTCCA	GGGATCCTC	CCGACCCAGG	GATTGAACCG	GCATCTCCTG	9900
TATTGGCAAG	TGGATTCTTT	ACCACTGTGC	CACCAAGGAA	GCCCCTGTTA	CTCTCTATGT	9960
CCCACTTAAT	TACCAAAGCT	GCTCCAAAGAA	AAAAGCCCTG	TCCCCTCTGA	GCTTCCCAGC	10020
CTGCAGAGGG	TGGTGGGGGT	AGACTGTGAC	CTGGGAACAC	CCTCCCCTTT	CAGGACTCCC	10080
GGGCCACGTG	ACCCACAGTC	CTGCAGACAG	CCGGTAGCT	CTGCTCTTCA	AGGCTCATTA	10140
TCTTTAAAAA	AAACTGAGGT	CTATTTTGTG	ACTTCGCTGC	CGTAACTTCT	GAACATCCAG	10200
TGCGATGGAC	AGGACCTCCT	CCCCAAGCCT	CAGGGGCTTC	AGGGAGCCAG	CCTTCACCTA	10260
TGAGTCACCA	GACACTCGGG	GGTGGCCCCG	CCTTCAAGGT	GCTCACAGTC	TTCCCATCGT	10320
CCTGATCAAA	GAGCAAGACC	AATGACTTCT	TAGGAGCAAAG	CAGACACCCA	CAGGACACTG	10380
AGGTTACCA	GAGCTGAGCT	GTCTTTTGA	ACCTAAAGAC	ACACAAGCTCT	CGAAGGTTTT	10440
CTCTTTAATC	TGGATTTAAG	GCCTACTTGC	CCCTCAAGAG	GGAAAGACAGT	CCTGCATGTC	10500
CCCAGGACAG	CCACTCGGTG	GCATCCGAGG	CCACTTAGTA	TTATCTGACC	GCACCCTGGA	10560
ATTAACTGGT	CCAAACTGGA	CAAAAACCTT	GGTGGGAAGT	TTCATCCCAAG	AGGCCTCAAC	10620
CATCCTGCTT	TGACCACCCT	GCATCTTTTT	TTCTTTTATG	TGTATGCATG	TATATATATA	10680
TATATATTTT	TTTTTTTTTC	ATTTTTTGGC	TGTGCTGGCT	GTTGCTTCCA	GTTGGGTGCG	10740
CAGGCTTCTC	TCTAGTTTCT	CTCTAGTCTT	CTCTTATCAC	AGAAGCAATCT	CTAGACGATC	10800
GACGCGT						10807

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 47 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8

AATTCGGATC	GACGCGTCGA	CGATATACTC	TAGACGATCG	ACGCGTA	47
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(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

(B) CLONE: BLGAMP3

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9

TGGATCCCCT	GCCGGTGCCT	CTGG	24
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(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

(B) CLONE: BLGAMP4

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACGCGTCAT	CCTCTGTGAG	CCAG	24
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(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (v i i) IMMEDIATE SOURCE:
 (B) CLONE: ZC6839

- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACTACGTAGT

10

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 42 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (v i i) IMMEDIATE SOURCE:
 (B) CLONE: ZC6632

- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGACGCGGAT CCTACGTACC TGCAGCCATG TTTTCCATGA GG

42

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (v i i) IMMEDIATE SOURCE:
 (B) CLONE: ZC6627

- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGGGGCTTCGG CAAGCTTCAG G

21

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (v i i) IMMEDIATE SOURCE:
 (B) CLONE: ZC6521

- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCAAAGACT TACTTCCCTC TAGA

24

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (v i i) IMMEDIATE SOURCE:
 (B) CLONE: ZC6520

- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCATGAACGT CCGTGGTGG TTGTGCTACC

30

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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6519

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACCACGCGAC GTTCATGCTC TAAAACCGTT

3 0

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6518

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCTGCGGGAT CCTACGTACT AGGGGGACAG GGAAAGG

3 6

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6629

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGACGCGAAT TCTACGTACC TGCAGCCATG AAAAGGATGG TTCT

4 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6630

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGACGCGAAT TCTACGTACC TGCAGCCATG AAACATCTAT TATTO

4 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6625

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTGAGATTTT CAGATCTTGT C

2 1

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6626

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAGAATTACT GTGGCCTACC A

21

(2) INFORMATION FOR SEQ ID NO-22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6624

(x i) SEQUENCE DESCRIPTION: SBQ ID NO:22:

GCTGCGGAAT TCTACGTACT ATTGCTGTGG GAA

33

(2) INFORMATION FOR SEQ ID NO-23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: ZC6514

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGACGCGGAT CCTACGTACC TGCAGCCATG AGTTGGTCCT TGCAC

45

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: 206517

(x i) SEQUENCE DESCRIPTION: SBQ ID NO:24:

GTCTCTGGTA GCAACATACT A

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: 206516

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGTTTCTAG CCCTACTAGT AG

22

(2) INFORMATION FOR SEQ ID NO:26:

(i) **SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(v i i) IMMEDIATE SOURCE:

- (B) CLONE: zo6515

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGGTTTCTAG CCCTACTAGT AG

22

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AAGCTACGCG TCGATCGTCT AGAGTATATC GTCGACGCGT CGATCGG

47

We claim:

1. A method for producing biocompetent fibrinogen comprising: 30

providing a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen A α chain,

the DNA segment comprising genomic DNA encoding the A α chain;

a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen B β chain.

the DNA segment comprising genomic DNA encoding the B β chain; and

a third DNA segment encoding a secretion 35
signal operably linked to a heterologous fibrinogen γ chain.

the DNA segment comprising genomic DNA encoding the γ chain,

6. A method according to claim 1 wherein said first, second and third DNA segments are expressed under the control of a β -lactoglobulin promoter.

7. A method according to claim 1 wherein said introducing step comprises injecting said first, second and third DNA segments into a pronucleus of said fertilized egg.

8. A method according to claim 1 wherein said fibrinogen is human fibrinogen.

9. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 470 to nucleotide 8100.

10. A method according to claim 1 wherein said second DNA segment comprises a sequence of nucleotides as shown in SEQ ID NO: 3 from nucleotide 512 to nucleotide 8100.

11. A method according to claim 1 wherein said species into which said DNA segments is introduced is sheep.

12. A method of producing biocompetent fibrinogen comprising:

incorporating a first DNA segment encoding a secretion signal operably linked to an A α chain of fibrinogen into a β -lactoglobulin gene to produce a first gene fusion comprising a β -lactoglobulin promoter operably linked to the first DNA segment

, the DNA segment comprising genomic DNA encoding the A α chain;

incorporating a second DNA segment encoding a secretion signal operably linked to a B β chain of fibrinogen into a β -lactoglobulin gene to produce a second gene fusion comprising a β -lactoglobulin promoter operably linked to the second DNA segment

, the DNA segment comprising genomic DNA encoding the B β chain;

60 incorporating a third DNA segment encoding a secretion
 signal operably linked to a γ chain of fibrinogen into a
 β -lactoglobulin gene to produce a third gene fusion
 comprising a β -lactoglobulin promoter operably linked
 to the third DNA segment

, the DNA segment comprising
genomic DNA encoding the γ
chain,

wherein each of said first,
 second and third segments are of the same species;
 65 introducing said first, second and third gene fusions into
 the germ line of a non-human mammal so that said
 DNA segments are expressed in a mammary gland of

obtaining milk from said mammal or its female progeny;
and

13. A method according to claim 12 wherein said mammal is a sheep, pig, goat or cow.

15. A method according to claim 12 wherein the molar ratio of said first, second and third gene fusions introduced is within the range of 0.5-1:0.5-1:0.5-1.

16. A method according to claim 12 wherein said introducing step comprises injecting said first, second and third gene fusions into a pronucleus of a fertilized egg and inserting said egg into an oviduct of a pseudopregnant female to produce female offspring carrying said gene fusions in the germ line, wherein said egg and said pseudopregnant female are of the same species.

18. A method for producing biocompetent fibrinogen comprising:

providing a transgenic female non-human mammal carrying in its germline heterologous

DNA segments

encoding A α , B β and γ chains of fibrinogen, wherein said segments are expressed in a mammary gland of said mammal and biocompetent fibrinogen encoded by said segments is secreted into milk of said mammal: 30

collecting milk from said mammal; and

recovering said biocompetent fibrinogen from said milk.

19. A method according to claim 18 wherein said mammal is a sheep, pig, goat or cow.

20. A method according to claim 18 wherein said mammal is a sheep.

21. A transgenic non-human female mammal that produces recoverable amounts of biocompetent human fibrinogen in its milk, wherein said mammal comprises:

a first DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen A α chain.

the DNA segment comprising
heterologous genomic DNA
encoding the A α chain;

a second DNA segment encoding a secretion signal operably linked to a heterologous fibrinogen B β chain.

the DNA segment comprising heterologous genomic DNA encoding the B β chain;

and
a third DNA segment encoding a secretion signal operably
linked to a heterologous fibrinogen γ chain. 45

the DNA segment comprising
heterologous genomic DNA
encoding the v chain;

and
further wherein each chain is derived from the same species
and is operably linked to additional DNA segments required
for its expression in the mammary gland of a host female
mammal.

[illegible]

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• • • • •

FIGURE 1

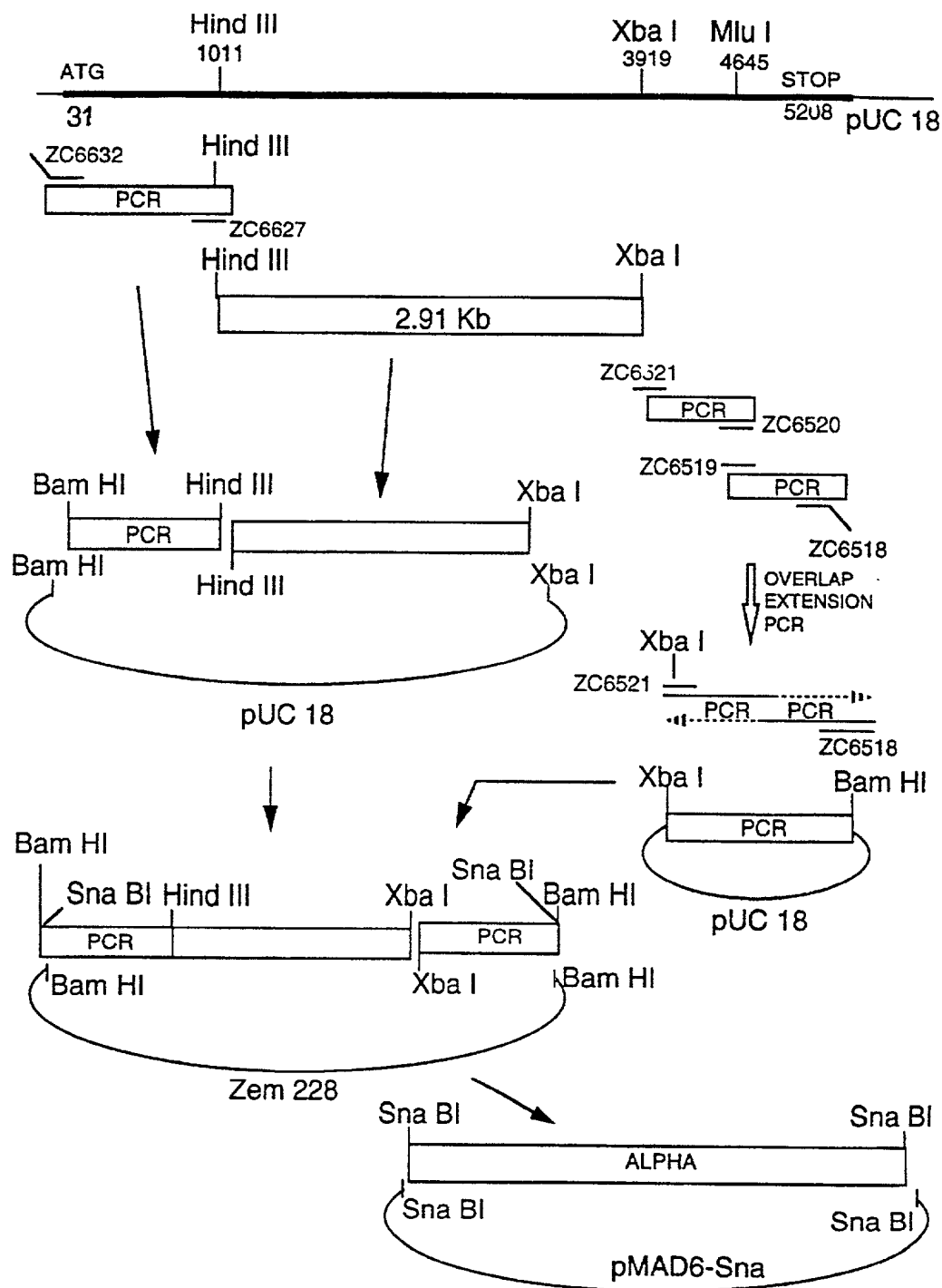


FIGURE 2

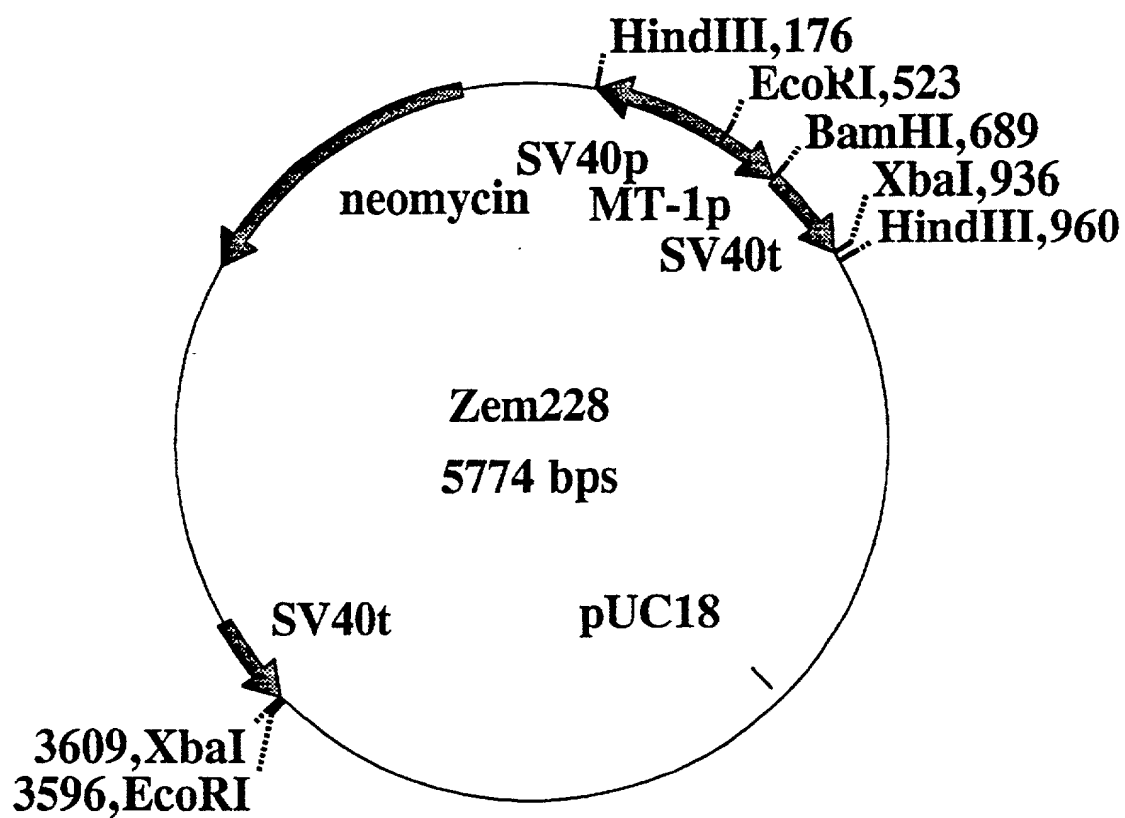


FIGURE 3

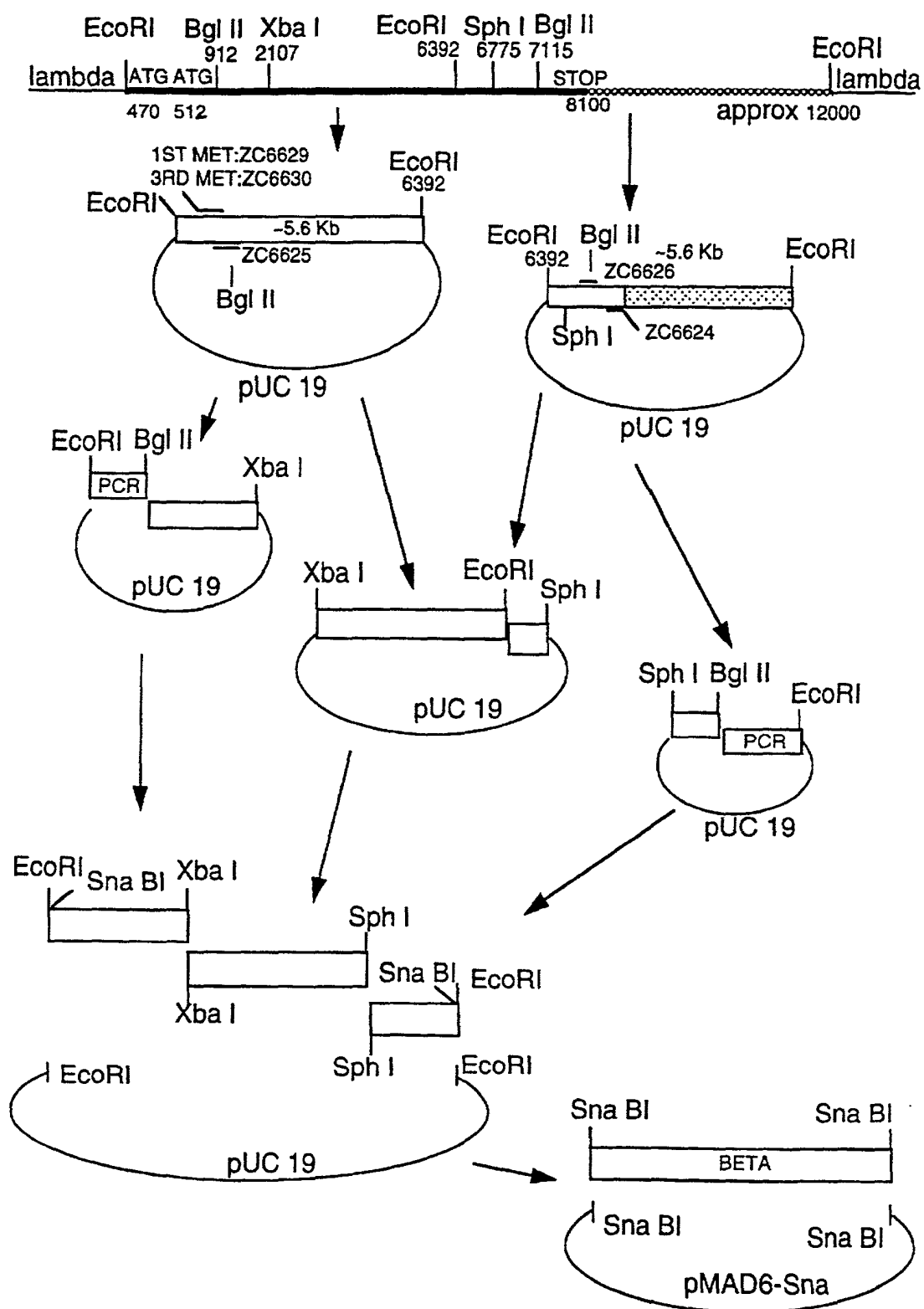


Figure 1 consists of 12 gel electrophoresis images, labeled (a) through (l). Each gel shows the results of PCR amplification of DNA from various bacterial strains. The gels are arranged in a vertical column. Each gel has multiple lanes, with some lanes labeled with numbers (1-12) and others with letters (A, B, C, D, E, F, G, H, I, J, K, L). The bands represent the amplified DNA products, with varying patterns of intensity and position across the different gels and lanes. For example, gel (a) shows a prominent band in lane 1, while gel (l) shows a band in lane 12. The patterns of bands across the different gels and lanes suggest that the PCR products are being used to identify or differentiate the bacterial strains.

FIGURE 4

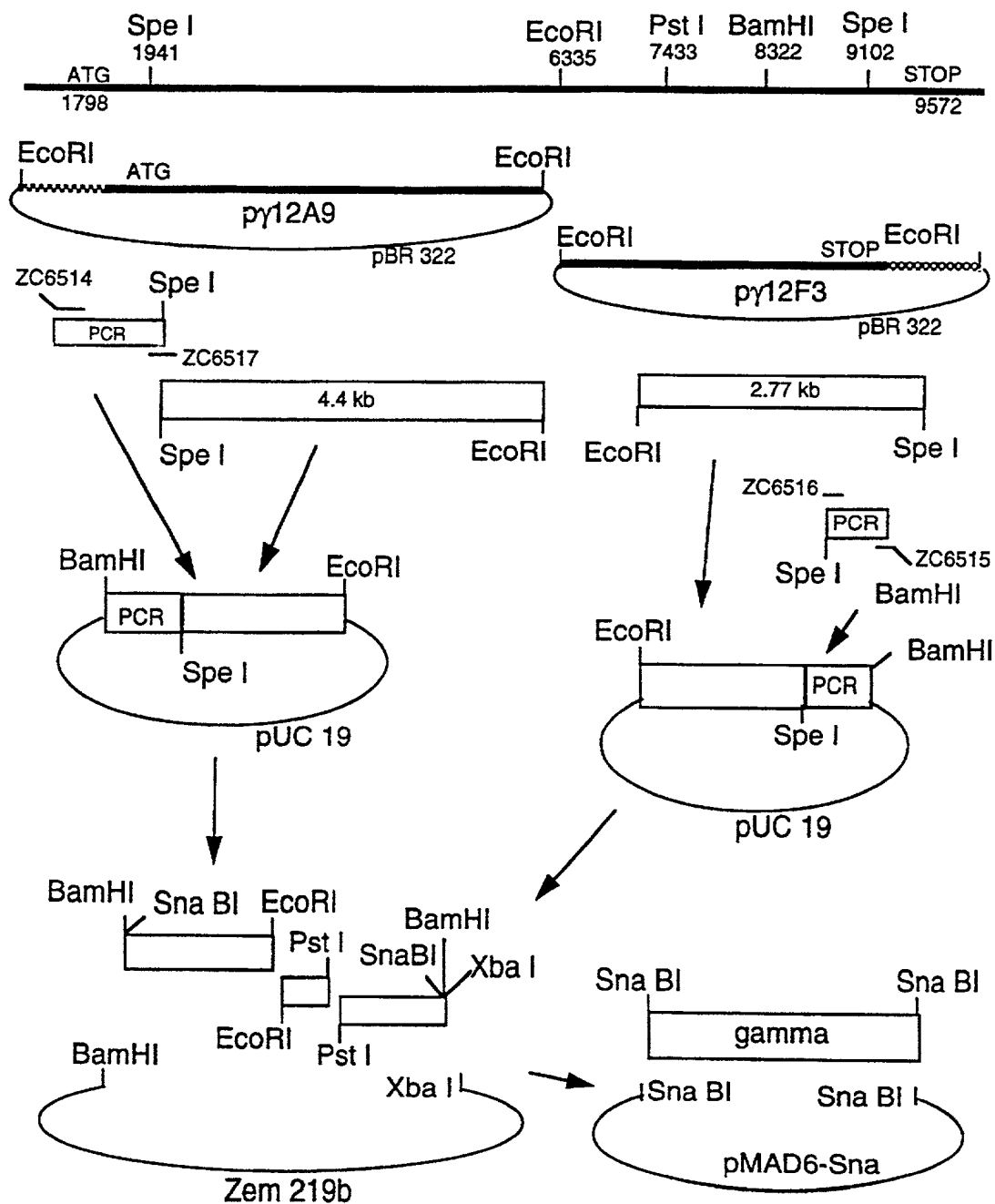
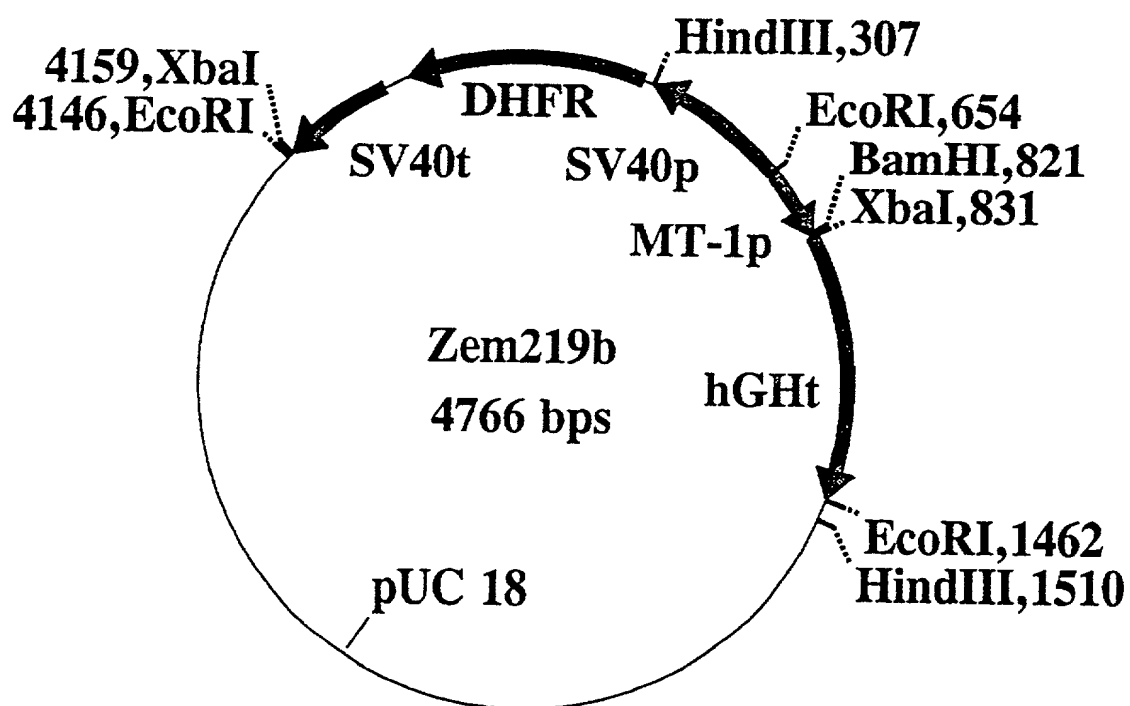


FIGURE 5



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reissue Application : Not yet assigned
Serial No. : Not yet assigned
Filed : January 15, 1999
Patent : 5,639,940
Patentee : Ian Garner, Michael A. Dalrymple,* Donna E. Prunkard
and Donald C. Foster
Assignee : Pharmaceutical Proteins, Ltd., and ZymoGenetics, Inc.
Issued : June 17, 1997
Application : 08/206,176
Filed : March 3, 1994
For : PRODUCTION OF FIBRINOGEN IN TRANSGENIC
ANIMALS

REISSUE DECLARATION AND POWER OF ATTORNEY

We, DONNA E. PRUNKARD and DONALD C. FOSTER, two of the four
named inventors of United States patent 5,639,940 ("the '940 patent"), and applicants for
reissue thereof, declare that:

1. We are citizens of the United States and have residences and post office
addresses as stated below under our respective names:

Donna E. Prunkard
1463 N.W. 92nd St.
Seattle, WA 98117

* The '940 patent incorrectly printed the middle initial of Dr. Dalrymple as "L".

Donald C. Foster
3002 NE 181st Street
Lake Forest Park, WA 98155

2. By concurrent Petition Under 37 C.F.R. § 1.324 and Motion Under 37 C.F.R. § 1.634, the four originally named inventors, Ian Garner, Michael A. Dalrymple, Donna E. Prunkard, and Donald C. Foster, have petitioned and moved to correct the inventorship for all of the claims of the '940 patent. As amended, Donna E. Prunkard and Donald C. Foster are the joint inventors of the subject matter claimed in the '940 patent.

3. We have reviewed and understand the contents of the '940 patent and this application for reissue thereof, including their claims, and believe that we are the original, first and joint inventors of the invention described and claimed in the '940 patent to the extent that a reissue application is being sought on that invention.

4. We make this declaration in support of this application for reissue of the '940 patent and acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to the patentability of the reissue application and its claims as defined in 37 C.F.R. § 1.56(a).

5. We do not know and do not believe that our invention was ever patented or described in any printed publication in any country before our invention thereof or more than one year prior to the March 3, 1994 filing date of our United States patent application 08/206,176, which issued as the '940 patent, or in public use or on sale in the United States more than one year prior to that filing date.

6. We believe that the '940 patent is "wholly or partly invalid" because of error, without deceptive intent, by reason of our having claimed more than we had a right to claim in view of the prior art. Specifically, all of the claims of the '940 patent recite methods and

transgenic animals employing "DNA" encoding, respectively, each of the A α , B β and γ chains of fibrinogen. To be patentable over the prior art, we believe that these claims should be limited to genomic DNA and thereby exclude cDNA.

7. In obtaining allowance of the claims of the '940 patent, we understand that our attorney argued that the claims were patentable over the prior art based on the unexpected production of useful amounts of biologically active, fibrinogen in the milk of transgenic animals. See Amendment, May 8, 1995, pp. 9-12. We understand that at the request of the Examiner and in view of that argument, our attorney then amended the claims to recite "biocompetent fibrinogen". See Office Action, Paper No. 13, May 21, 1996, p. 4; Amendment, October 1, 1996, pp. 1-2, 6-8. It was this amendment that we understood led to allowance of the claims and grant of the '940 patent.

8. We do not believe that such amendment renders the claims of the '940 patent patentable over the prior art. We believe at the time we made our invention, in view of art published more than one year before the filing date of the application that issued as the '940 patent that it would have been obvious to the skilled worker in the recombinant fibrinogen expression and transgenic arts that biocompetent fibrinogen would be produced in the milk of transgenic animals carrying the cDNAs encoding the A α , B β and γ chains of fibrinogen at about the same level on a per cell basis as had already been produced in mammalian cells transfected with those cDNAs, i.e., about 5 μ g/mg of cellular protein/day. Indeed, we understood that when such cDNAs were actually used to produce transgenic mice, those mice produced in their milk biocompetent fibrinogen at about that expected level.

9. We also believe that at the time we made our invention, in view of prior art published more than one year before we filed our application, that the skilled worker would

have reasonably expected that genomic DNAs encoding the A α , B β and γ chains of fibrinogen could be used to produce biocompetent fibrinogen in the milk of transgenic animals in the best case at that same level and potentially at much lower levels. We believe, however, that at the time we made our invention, in view of prior art published more than one year before we filed our application, it would not have been obvious to that skilled worker that those genomic DNAs could be used to produce biocompetent fibrinogen in the milk of transgenic animals with a reasonable expectation of success at levels on a per cell basis higher than those already produced in mammalian cells transfected with cDNAs encoding the three fibrinogen claims. It was to this unexpectedly high expression level that we understood our attorney was referring when he argued to the Examiner that our invention was patentable because it unexpectedly produced useful amounts of biocompetent fibrinogen.

10. We believe that the error described in paragraphs 7-9 above arose without deceptive intent. All of the examples in our application used genomic DNA encoding the A α , B β and γ chains of human fibrinogen. We understood that when our attorney made the above arguments to the Examiner and amended our claims to obtain their allowance he had in mind the genomic DNA as used in our Examples. See the '940 patent, Examples II, III, and IV. We understood that he failed to consider that the claims literally included cDNA.

11. We also believe that the '940 patent is also "wholly or partly inoperative" because of error, without deceptive intent, by reason of our having claimed less than we had a right to claim in view of the disclosure of the application that issued as the '940 patent. Specifically, none of the patent claims is directed to a set comprising a first, second, and third DNA segment encoding a secretion signal operably linked to the heterologous fibrinogen A α , B β and γ chains, respectively, each of the DNA segments comprising genomic DNA encoding

the respective fibrinogen chain, and wherein each of the chains is from the same species and is operably linked to additional DNA segments required for expression in the mammary glands of a host female mammal. This set of DNA segments is useful in the methods of claims 1-20 and 23-25 of the '940 patent and in producing the non-human mammals of claims 21-22 and 26-33 of the '940 patent. This set of DNA segments is also described and enabled in the '940 patent and should have been claimed in that patent and in the application from which it issued. Without a claim to this set of DNA segments, we understand that a third party might potentially prepare the recited set of DNA segments in the United States and export that set from the United States for use outside the United States in the production of fibrinogen in the milk of transgenic animals. We also understand that without reissue such acts would not infringe the claims of the '940 patent.

12. We understand and believe that the error described in paragraph 11 arose without deceptive intent.

13. We understand and believe that these errors in the claims of the '940 patent were discovered while reviewing the '940 patent, with counsel, in connection with Garner v. Velander, Interference 104,242. We have filed this reissue application promptly after recognizing the errors.

14. As named inventors, we hereby appoint the following attorneys and agents in connection with the '940 patent and this application for reissue thereof, with full power to prosecute this application for reissue and to transact all business in the United States Patent and Trademark Office in connection therewith and with the '940 patent:

James F. Haley (Reg. No. 27,794)

Karen Mangesarian (Reg. No. p43,772)

Z. Ying Li (Reg. No. 42,800)

all of Fish & Neave, 1251 Avenue of the Americas, 49th Floor, New York, NY 10020

Send correspondence to: James F. Haley, Jr.
1251 Avenue of the Americas
New York, New York 10020-1104

Direct telephone calls to: James F. Haley
(212) 596-9000

15. We hereby declare that we understand the English language, and that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application for reissue or any Patent issued thereon.


DONNA E. PRUNKARD

Date: 1-13-99


DONALD C. FOSTER

Date: 1-13-99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Reissue Application : Not yet assigned

Serial No. : Not yet assigned

Filed : January 15, 1999

Patent : 5,639,940

Patentee : Ian Garner, Michael A. Dalrymple,* Donna E. Prunkard and Donald C. Foster

Assignee : Pharmaceutical Proteins, Ltd., and ZymoGenetics, Inc.

Issued : June 17, 1997

Application : 08/206,176

Filed : March 3, 1994

For : PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS

DECLARATION IN SUPPORT OF REISSUE APPLICATION

I, GARY E. PARKER, declare that:

1. I make this declaration in support of the accompanying Reissue Application for United States patent 5,639,940.
2. I am Principle Patent Agent at ZymoGenetics, Inc., Seattle, Washington. In March 1994, I was the Manager of the Patent Department at ZymoGenetics. I am registered to practice before the United States Patent and Trademark Office. I am not an attorney.

* The '940 patent incorrectly printed the middle initial of Dr. Dalrymple as "L".

3. I prepared and filed the '176 application that issued as the above-identified '940 patent.

4. I understand and believe that the '940 patent is "wholly or partly invalid" because of error, without deceptive intent. I believe that the patent claims more than the inventors had a right to claim in view of the prior art. Particularly, all of the claims of the '940 patent recite methods and transgenic animals employing "DNA" encoding, respectively, each of the A α , B β and γ chains of fibrinogen. I believe that these claims should be limited to genomic DNA, and thereby exclude cDNA, to be patentable over the prior art.

5. In obtaining allowance of the claims of the '940 patent over the prior art, I argued that the claims were patentable because production of useful amounts of biologically active, fibrinogen in the milk of transgenic animals was unexpected. See Amendment, May 8, 1995, pp. 9-12. At the request of the Examiner and in view of that argument, I amended the claims to recite "biocompetent fibrinogen". See Office Action, Paper No. 13, May 21, 1996, p. 4; Amendment, October 1, 1996, pp. 1-2, 6-8. I believe that it was this amendment that led to allowance of the claims and grant of the '940 patent.

6. I do not now believe that my amendment renders the claims of the '940 patent patentable. I understand and believe that at the time the inventors made their invention in view of art published more than one year before the filing date of the application that issued as the '940 patent, it would have been obvious to the skilled worker in the recombinant fibrinogen expression and transgenic arts that biocompetent

human fibrinogen would be produced in the milk of transgenic animals carrying the cDNAs encoding the A α , B β and γ chains of human fibrinogen at about the same level on a per cell basis as had already been produced in mammalian cells transfected with those cDNAs, i.e., about 2 μ g/2x10⁶cells/day.* In fact, when cDNAs encoding the three chains of fibrinogen were used to produce transgenic mice, those mice produced biocompetent fibrinogen in their milk at about the expected level.

7. I also understand and believe that at the time of invention, in view of prior art published more than one year before the application was filed, that it would not have been obvious to that skilled worker that genomic DNAs encoding the A α , B β and γ chains of fibrinogen could be successfully used to produce biocompetent fibrinogen in the milk of transgenic animals at levels on a per cell basis higher than those already produced in mammalian cells using the corresponding cDNAs. It was to this unexpectedly higher production of biocompetent fibrinogen which I referred when I argued that the Garner claims were patentable. *See, supra*, ¶ 5.

8. I believe that the error described in paragraphs 4-7 above arose without deceptive intent. Although I do not recall the specific details of the prosecution of the application that issued as the '940 patent, I believe that during prosecution I did not consider that the claims covered low level expression of cDNA. All of the examples in the application used genomic DNA encoding the A α , B β and γ chains of human fibrinogen. See the '940 patent, Examples II, III, and IV. And, it was only genomic DNA that the inventors had used to produce useful amounts of biocompetent fibrinogen in the milk of

* During prosecution of the application that issued as the Garner '940 patent, the Examiner cited Roy et al. for its report of the production of 2 μ g of fibrinogen per 2x10⁶cells/day in COS cells.


transgenic animals. It was this work that was the basis of my patentability arguments. As best I can recollect, I failed to consider or realize that the pending claims literally included cDNA.

9. I also understand and believe that the '940 patent is also "wholly or partly inoperative" because of error, without deceptive intent. I believe that the patent claims less than the inventors had a right to claim in view of the disclosure of the application that issued as the '940 patent. None of the patent claims is directed to a set comprising a first, second, and third DNA segment encoding a secretion signal operably linked to the heterologous fibrinogen A α , B β and γ chains, respectively, each of the DNA segments comprising genomic DNA encoding the respective fibrinogen chain, and wherein each of the chains is from the same species and is operably linked to additional DNA segments required for expression in the mammary glands of a host female mammal. This set of DNA segments is useful in the methods of claims 1-20 and 23-25 of the '940 patent and in producing the non-human mammals of claims 21-22 and 26-33 of the '940 patent. I also believe that this set of DNA segments is described and enabled in the '940 patent. Finally, I believe that the set of DNA segments should have been claimed in the '940 patent and in the application from which it issued. Without a claim to this set of DNA segments, I understand and believe that a third party might potentially prepare the recited set of DNA segments in the United States and export that set from the United States for use outside the United States in the production of fibrinogen in the milk of transgenic animals. I also understand and believe that without reissue such acts would not infringe the claims of the '940 patent.

10. The error described in paragraph 11 arose without deceptive intent. Today, I do not recall the specific details of the prosecution of the patent application that issued as the '940 patent. Nonetheless, to the best of my recollection, I believe that I did not claim the set of DNA segments now claimed in reissue claim 34, because I was focusing on the end product -- fibrinogen -- and the methods and animals used to make it. I did not, as I recall, consider claiming the various intermediates -- including this set of DNA segments -- used in producing those animals or the claimed method.

11. I discovered these errors (paragraphs 4-7 and 8-10) in the claims of the '940 patent while reviewing the '940 patent, with counsel, in connection with Garner v. Velander, Interference 104,242.

12. I hereby declare that I understand the English language, and that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application for reissue or any Patent issued thereon.


GARY E. PARKER

Date: Jan. 13, 1999